(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 September 2002 (12.09.2002)

PCT

(10) International Publication Number
WO 02/070683 A2

(51) International Patent Classification[7]: C12N 13/00, 1/16, 1/04, C12P 1/02, A62D 3/00, B09B 3/00 // (C12P 1/02, C12R 1:645)

(21) International Application Number: PCT/GB02/00915

(22) International Filing Date: 1 March 2002 (01.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

09/797,371	1 March 2001 (01.03.2001)	US
09/797,372	1 March 2001 (01.03.2001)	US
09/797,377	1 March 2001 (01.03.2001)	US
09/797,378	1 March 2001 (01.03.2001)	US
09/797,381	1 March 2001 (01.03.2001)	US
09/797,382	1 March 2001 (01.03.2001)	US
09/797,437	1 March 2001 (01.03.2001)	US
09/797,493	1 March 2001 (01.03.2001)	US

(71) Applicant *(for all designated States except US)*: **ULTRA BIOTECH LIMITED** [GB/GB]; 60 Circular Road, 2nd Floor, Douglas IM1 1SA (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **CHEUNG, Ling, Yuk** [CN/CN]; 2 Dai Fu Street, Tai Po Industrial Estate, New Territories, Hong Kong (CN).

(74) Agents: **McCALLUM, William, Potter** et al.; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *without international search report and to be republished upon receipt of that report*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 02/070683 A2

(54) Title: BIOLOGICAL COMPOSITIONS FOR SOLID WASTE TREATMENT

(57) Abstract: The present invention relates to biological compositions useful for the treatment of solid waste. The biological compositions of the invention comprises a plurality of yeast cells which is capable of suppression of growth of pathogenic microorganisms, breakdown of undesirable chemicals, such as antibiotics, insecticides and waste chemicals, and reducing the odor of organic waste matters. The yeast cells of the invention are produced by culturing the plurality of yeast cells under activation conditions in the presence of a series of electromagnetic fields. The invention also relates to methods for manufacturing the treatment composition.

BIOLOGICAL COMPOSITIONS FOR SOLID WASTE TREATMENT

1. FIELD OF THE INVENTION

The invention relates to biological compositions that comprise yeasts for the treatment of solid waste. The yeasts in the compositions of the invention have been stimulated to perform a variety of functions including degradation of chemicals, reduction of odor and suppression of microorganisms. The invention also relates to methods for manufacturing the biological compositions, and methods for using the biological compositions to treat waste.

2. BACKGROUND OF THE INVENTION

Large amounts of solid waste are generated daily by industrial and agricultural activities, and by municipalities. If the waste is not treated properly, it can cause severe and long-lasting damage to the environment. In 1995-1996, 208 million tons of municipal solid waste were generated in the United States. Of the municipal solid waste generated, 56 million tons (27 percent) were recovered by recycling or composting, 33.5 million tons (16 percent) were combusted at high temperatures, and 118.5 million tons (57 percent) were landfilled.

Municipal waste may be treated instead of landfilled. One type of municipal waste treatment involves high temperature burning of the waste in an incinerator. This combustion of municipal waste significantly reduces its volume. The ash from municipal waste combustion must be properly managed to prevent the environmental damage from any potential hazardous constituents. Also, emissions from the incinerator's smoke stack must be within acceptable regulatory levels.

In the agricultural area, despite the importance of mineral fertilizers in providing mankind with abundant agricultural products, the harm done to the environment has been recognized in recent years. Mineral fertilizers may incurred damages to soils. For example, most nitrogen fertilizers may acidify soils, thereby adversely affecting the growth of plants and other soil organisms. Extensive use of chemical nitrogen fertilizers may also inhibit the activity of natural nitrogen fixing microorganisms, thereby decreasing the natural fertility of soils. The long term use of mineral fertilizers may also cause severe environmental pollution. For example, the loss of nitrogen and phosphate fertilizers due to leaching and soil erosion has led to contamination of soil and ground water, and eutrophication of surface water.

Another type of agricultural waste is manure which, if not stored or disposed of properly, can pose health and environmental threats. For example, it can cause air pollution, i.e., odor and dust; and contamination of surface and ground water with excess nutrients, organic matter, salts, and pathogens. For example, manure contains pathogenic microorganisms, such as *Escherichia coli, Salmonella spp.*, and *Shigella spp.*

Overall, cleaning up pollution as a result of a poor waste management strategy has been a complicated and difficult task. The cost for such a task is also astronomical. Thus, there is a need for inexpensive and effective methods to handle the waste generated by the myriad of human activities.

The use of biological compositions in pollution control has been proposed for many situations. Biological fertilizers utilizing microorganisms have been proposed as alternatives to mineral fertilizers. Naturally occurring nitrogen fixing microorganisms including bacteria, such as *Rhizobium*, *Azotobacter*, and *Azospirillum*, (See for example, U. S. Patent No. 5,071,462) and fungi, such as *Aspergillus flavus-oryzae*, (See, for example, U. S. Patent No. 4,670,037) have been utilized in biological fertilizers. Naturally occurring microorganisms capable of solubilizing phosphate rock ore or other insoluble phosphates into soluble phosphates have also been utilized in biological fertilizers either separately (e.g., U. S. Patent No. 5,912,398) or in combination with nitrogen fixing microorganisms (e.g., U. S. Patent No. 5,484,464). An approach based on recombinant DNA techniques has been developed to create more effective nitrogen fixing, phosphorus decomposing, and potassium decomposing bacterial strains for use in a biological fertilizer, see, for example, U.S. Patent No. 5,578,486; PCT publication WO 95/09814; Chinese patent publication: CN 1081662A; CN 1082016A; CN 1082017A; CN 1103060A; and CN 1109595A.

Citation of documents herein is not intended as an admission that any of the documents cited herein is pertinent prior art, or an admission that the cited documents are considered material to the patentability of the claims of the present application. All statements as to the date or representations as to the contents of these documents are based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.

3. SUMMARY OF THE INVENTION

The present invention relates to biological compositions useful for the treatment of solid waste. The biological compositions of the invention comprises a plurality of yeast cells which is capable of suppression of growth of pathogenic microorganisms,

breakdown of undesirable chemicals, such as antibiotics, insecticides and waste chemicals, and reducing the odor of organic waste matters.

In various embodiments, the present invention uses yeasts that are commercially available and/or accessible to the public, such as but not limited to *Saccharomyces cerevisiae*. The yeast cells of the invention are produced by culturing the plurality of yeast cells under activation conditions in the presence of a series of electromagnetic fields, such that the yeast cells become highly efficient in performing certain metabolic functions. Accordingly, the invention also relates to methods for manufacturing the treatment composition comprising culturing the yeast cells under activation conditions, mixing various yeast cell cultures of the present invention, followed by drying the yeast cells and packing the final product.

In particular, the invention encompasses methods for treatment of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the antibiotics in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The antibiotics that can be degraded by the yeast cells of invention include are not limited to penicillin, chlortetracycline, oxytetracycline, doxycycline, tetracycline, streptomycin, kanamycin, erythromycin, spiramycin, bacitracin, colistin, chloramphenicol, cephalothin, neomycin and novobiocin.

The invention also encompass methods for treatment of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The undesirable chemicals that can be degraded by the yeast cells of the invention include but are not limited to toluene, ethylbenzene, trichlorophenols, dimethylbenezenes, benzaldehyde, propylaldehyde, nenanthaldehyde, dichlorobenzenes, acetophenone, arsanilic acid, roxarsone, furazolidonum, decoquinate, trichlorophonum, dinitomide, dichlorvos, momocrotophos, dimethoate, DDT and toxaphene. The undesirable chemicals also include organic and inorganic salts such as ammonium compounds, nitrites or nitrates, and phosphates.

The invention further encompasses methods for reducing the odor of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of odorous molecules in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of

electromagnetic fields having specific ranges of frequencies and field strengths. The odorous molecules include but are not limited to hydrogen sulfide, ammonia, indole, skatol, acetic acid, methylamine, and p-cresol.

The invention further encompasses methods for suppressing the growth of pathogenic bacteria in solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to suppress the growth of pathogenic bacteria in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The pathogenic bacteria are selected from the group consisting of *Staphylococcus aureus, Diplococcus pneumoniae, Bacillus anthracis, Mycobacterium tuberculosis, Salmonella* species, *E. coli, Vibrio* species, *Shigella* species, *Clostridium botulinum*, and *Bacillus aerogenes capsulatus*.

The methods of the invention can be carried out using combinations of the yeast cells in the treatment of solid waste. A biological composition of the invention is added to the solid waste, said biological composition comprising at least one of the following yeast cell components (a) a first yeast cell component comprising a plurality of yeast cells that degrade antibiotics in solid waste; (b) a second yeast cell component comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste; (c) a third yeast cell component comprising a plurality of yeast cells that reduce the amount of odorous molecules in solid waste; and (d) a fourth yeast cell component comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in the solid waste. The amount of time for the treatment can be determined empirically by monitoring the change in levels of the antibiotics, undesirable chemicals, pathogenic bacteria, and malodorous molecules in the solid waste, and can range from several hours, several days, and up to two or more weeks.

The invention further include methods for using the biological compositions of the present invention for the management, storage, processing, recycling or disposal of solid waste.

4. BRIEF DESCRIPTION OF FIGURES

Fig. 1 Activation of yeast cells. 1 yeast cell culture; 2 container; 3 electromagnetic field source.

Fig. 2. Adaptation of yeast cells to a soil type. 4 input electrode; 5 container; 6 electrode; 7 yeast cell culture; 8 electromagnetic field source; 9 temperature controller.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides biological compositions that comprise yeast cells. The present invention also provides methods for manufacturing the biological compositions as well as methods for using the biological compositions.

The biological compositions of the invention are useful for the treatment of solid waste so as to reduce the health risk and its impact on the environment that are often associated with its storage, transportation, processing, recycling and/or disposal. The use of such compositions may lower the overall cost of managing solid waste for a community, a business, or a farm, and make feasible the recycling of certain types of solid waste. As used herein, treatment of a solid waste refers to a process which changes the physical, chemical, or biological character(s) of the solid waste that make the solid waste less objectionable while it is being stored, transported, recycled, handled, or less of a health threat or an environmental threat, than if the solid waste is not treated. The treatment generally renders the waste less hazardous, or makes the solid waste safer to transport, store, handle or recycle.

According to the invention, the biological compositions comprise a plurality of yeast cell components. Each yeast cell component is a population of yeast cells which comprises a plurality of yeast cells that are capable of performing one or more desired functions falling within the following categories: (1) suppression of growth of pathogens, (2) degradation of undesirable chemicals, or (3) reducing the odor of organic materials.

In one embodiment, a biological composition of the invention comprises at least one yeast cell component that can perform a function in one of the three categories. In preferred embodiments, the biological compositions of the invention comprises yeast cell components that provide functions in all three categories. Thus, the preferred biological fertilizer compositions comprise at least three different yeast cell components. It will be understood that different alternative formulations of yeast cell components are contemplated.

As used herein, the term "solid waste" broadly refers to any kind of material that is discarded because it has served its purpose or it is a by-product that is of no use, and that includes physiological waste excreted by humans and animals. The sources of solid

waste include residential, commercial, agricultural and industrial activities. Non-industrial and non-agricultural solid waste such as trash or garbage collected from urban areas, contains discarded food materials or materials used in food preparation, and other assorted dry materials, such as paper, fabric, or plastics. Especially in residential areas and commercial areas which have restaurants and hotels, the predominant type of solid waste, herein referred to as "garbage", comprises mainly decomposable food wastes. Garbage which supports growth of pathogenic organisms and becomes malodorous due to decay, can be efficiently treated by the biological compositions of the invention. The type of solid waste that lends itself to treatment by the biological compositions of the invention has the characteristic of a high organic content.

Another type of solid waste that can be treated by the compositions of the invention is sludge. The term "sludge" as used herein broadly encompasses any solid matter that has settled out of suspension in the course of sewage storage and/or treatment, for example but not limited to, residues in a waste lagoon, residues in an urban sewage treatment plant, or sewage concentrate. The term "sludge" also include semi-solid matters, and mixtures of effluent and sediments. The term thus encompasses sludge having a wide range of viscosity, density, and water content, as well as sludge which has been partially processed or stabilized. Depending on the source, sludge may contain a variety of undesirable chemicals that may have an adverse impact on the environment if it is not properly treated. Sludge is malodorous, and supports growth of pathogenic organisms.

The biological compositions of the invention can also treat waste products produced as a result of agricultural activities. Typically, the waste is produced by animals in operations such as but not limited to ranches, farms, slaughterhouses, and markets. The continuous production and accumulation of large amounts of animal excrement creates a malodorous environment, and poses a health risk to humans and livestock due to the presence of pathogenic microorganisms. Agricultural waste can also contain undesirable chemicals, such as antibiotic feed additives, chemical fertilizers, pesticides, and herbicides, that may pollute the environment if the waste is not managed properly.

The term "animal manure" as used herein broadly encompasses organic material that comprises the feces and urine of feedlot animals with or without accompanying litter such as straw, hay, or bedding, that is traditionally used to fertilize land. Poultry manure includes but are not limited to manure produced by domesticated birds, such as chicken, duck, turkey, goose, quail, squab, ostrich, and the like. Poultry manure include excrement or guano produced by non-domesticated bird species. Cattle manure as used herein encompasses waste from domesticated ruminant mammals, such as dairy cows, or

beef cattle. The term "cattle manure" as used herein is not limited to just cattle but include other animals that graze, and that are kept primarily for their milk, meat, skin, hair, and pelts. Cattle manure includes but are not limited to manure produced by buffalos, bisons, yaks, horses, donkeys, mules, sheep, goats, camels, and the like. Cattle manure also include excrement produced by non-domesticated herds. The term "swine manure" as used herein includes but are not limited to manure produced by swines, hogs, pigs, and the like. Other agricultural waste include field crop residues, bagasse, waste from fruit and vegetable packing facilities, waste from animal product packing facilities which include animal carcasses.

In various embodiments, the biological compositions of the invention are particularly useful in treating garbage, sludge, and manure.

Municipal waste in many cases is temporarily stored at waste transfer stations. At the transfer station, waste is off-loaded from local collection routes and in some cases sorted according to type. The waste is then loaded onto larger trucks or rail cars for transport to either a municipal waste treatment or disposal facility. Generally, depending on the source of the solid waste, glass, metal, wood, and other inorganic or non-decomposable items are separated from waste of a high organic content through sorting and separating operations. These can be carried out by methods well known in the recycling / garbage disposal industry, such as mechanically, using differences in such physical characteristics of the solid waste as size, and density. Shredding or pulverizing can reduce the size of the waste articles to fine particles, resulting in a uniform mass of material which can be more easily handled, e.g., mixing or transport. Due to the variation of constituents in manure, sludge, or garbage, it may be desirable to subject a sample of a batch of waste material to analysis to determine the amount and type of pathogenic organisms and undesirable chemicals present in the batch.

While the following terms are believed to have well-defined meanings in the art, the following are set forth to facilitate explanation of the invention.

As used herein, the phrase "suppressing the growth of pathogens" refers to a decrease or lack of increase in the number of pathogenic microorganisms present in a sample of solid waste over a period of time, as a result of the presence of the yeast cells of the invention in the sample. It is to be understood that in the absence of the yeast cells, the number of pathogens in the sample would increase naturally. Many such microorganisms cause diseases in humans and animals, and may include bacteria such as *Escherichia* species, *Salmonella* species, *Shigella* species, *Mycobacterium* species, *Staphylococcus* species, *Bacillus* species, *Streptococcus* species, and *Diplococcus* species.

As used herein, the phrase "degradation of undesirable chemicals" refers to biological or biochemical processes which result in the conversion of chemical compounds that are undesirable, e.g., environmental toxins, in solid waste to an inactive form, such as the breakdown of such compounds into lower molecular weight compounds. Antibiotics are commonly present in manure and such compounds are not desired in a fertilizer made from manure because of the potential risk of ingestion by humans, for example, by eating vegetables grown using a fertilizer comprising contaminated organic material, and the possible spread of antibiotic resistance in the environment. Many antibiotics are added to animal feed to protect various farm animals, such as chicken, turkey, and swine, from bacterial and parasitic diseases, and to promote growth. A significant amount of antibiotic feed additive is excreted by the animals, and thus accumulates in manure and sludge. Many kinds of antibiotics have been used in animal operations, such as but not limited to aminoglycosides, tetracyclines, beta-lactams, glycopeptides, and macrolides. Examples of antibiotics approved for use in farms in United States include but are not limited to, bacitracin methylene disalicylate, bacitracin zinc, bambermycins, oxytetracycline, chlortetracycline, penicillin, tylosin/sulfamethazine, roxarsone, nitrasone, monensin, lasalocid, carbodox, tiamulin, hygromycin B, nystatin, novobiocin, sulfadimethoxine, ormetroprim, lincomycin, fenbendazole, and virginiamycin. The presence and quantity of such antibiotics in a composition can be determined by any methods known in the art, for example, high performance liquid chromatography (HPLC).

As used herein, the phrase "reducing the odor of organic materials" refers to a process which results in a lower concentration of one or more odorous compounds in the organic materials present in solid waste. Odorous compounds, such as but not limited to hydrogen sulfide, ammonia, indole, skatole (i.e, 3-methyl-1H-indole), p-cresol, and organic acids, are known to contribute to the malodorous quality of solid waste. The concentration of such malodorous compounds in, for example, poultry manure or in a sample of air in contact with the manure can be determined by any method well known in the art, including but not limited to gas chromatography, or mass spectrometry. Odor is a perception of smell by an organism with olfactory organs. A reduction of the intensity of the odor associated with solid waste can be determined subjectively. Various methods and techniques are known to measure the intensity of an odor. One subjective measurement of odor intensity is to measure the dilution necessary so that the odor is imperceptible or doubtful to a human or animal test panel. Alternatively, a recognition threshold may also be used which is a higher concentration at which the character of the odor is recognized. Any methods and

techniques for objectively or subjectively determine the intensity of an odor can be used to monitor the performance of the compositions and methods of the invention.

The inventor discovered that, under certain culture conditions, yeasts can be activated to become highly efficient in certain metabolic functions which result in an ability of the activated yeasts to suppress growth of pathogens, degrade undesirable chemicals, or reduce the odor of organic materials.

According to the invention, a yeast cell component of the biological fertilizer composition is produced by culturing a plurality of yeast cells in an appropriate culture medium in the presence of an alternating electromagnetic field or multiple alternating electromagnetic fields in series over a period of time. The culturing process allows yeast spores to germinate, yeast cells to grow and divide, and can be performed as a batch process or a continuous process. As used herein, the terms "alternating electromagnetic field", "electromagentic field" or "EM field" are synonymous. An electromagnetic field useful in the invention can be generated by various means well known in the art. A schematic illustration of exemplary setups are depicted respectively in Fig. 1. An electromagnetic field of a desired frequency and a desired field strength is generated by an electromagnetic wave source (3) which comprises one or more signal generators that are capable of generating electromagnetic waves, preferably sinusoidal waves, and preferably in the frequency range of 30 MHz - 3000 MHz. Such signal generators are well known in the art. Signal generators capable of generating signal with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output signal, and thus the strength of the EM field.

The electromagnetic field can be applied to the culture by a variety of means including placing the yeast cells in close proximity to a signal emitter connected to a source of electromagnetic waves. In one embodiment, the electromagnetic field is applied by signal emitters in the form of electrodes that are submerged in a culture of yeast cells (1). In a preferred embodiment, one of the electrodes is a metal plate, and the other electrode comprises a plurality of wires configured inside the container (2) so that the energy of the electromagnetic field can be evenly distributed in the culture. The number of electrode wires used depends on both the volume of the culture and the diameter of the wire. For example, for a culture having a volume of 5000 ml, one electrode wire having a diameter of between 0.1 to 1.2 mm can be used for each 100 ml of culture; for a culture having a volume greater than 1000 l, one electrode wire having a diameter of between 3 to 30 mm can be used for each 1000 l of culture. See Figure 1.

Without being bound by any theory or mechanism, the inventor believes that the culture conditions activate and/or enhance the expression of a gene or a set of genes in a yeast cell such that the cell becomes more efficient in performing certain metabolic activities which lead to the respective desired results.

In various embodiments, yeasts of the genera of *Saccharomyces, Schizosaccharomyces, Sporobolomyces, Torulopsis, Trichosporon, Wickerhamia, Ashbya, Blastomyces, Candida, Citeromyces, Crebrothecium, Cryptococcus, Debaryomyces, Endomycopsis; Geotrichum, Hansenula, Kloeckera, Lipomyces, Pichia, Rhodosporidium,* and *Rhodotorula* can be used in the invention.

Non-limiting examples of yeast strains include *Saccharomyces cerevisiae* Hansen, ACCC2034, ACCC2035, ACCC2036, ACCC2037, ACCC2038, ACCC2039, ACCC2040, ACCC2041, ACCC2042, AS2.1, AS2.4, AS2.11, AS2.14, AS2.16, AS2.56, AS2.69, AS2.70, AS2.93, AS2.98, AS2.101, AS2.109, AS2.110, AS2.112, AS2.139, AS2.173, AS2.174, AS2.182, AS2.196, AS2.242, AS2.336, AS2.346, AS2.369, AS2.374, AS2.375, AS2.379, AS2.380, AS2.382, AS2.390, AS2.393, AS2.395, AS2.396, AS2.397, AS2.398, AS2.399, AS2.400, AS2.406, AS2.408, AS2.409, AS2.413, AS2.414, AS2.415, AS2.416, AS2.422, AS2.423, AS2.430, AS2.431, AS2.432, AS2.451, AS2.452, AS2.453, AS2.458, AS2.460, AS2.463, AS2.467, AS2.486, AS2.501, AS2.502, AS2.503, AS2.504, AS2.516, AS2.535, AS2.536, AS2.558, AS2.560, AS2.561, AS2.562, AS2.576, AS2.593, AS2.594, AS2.614, AS2.620, AS2.628, AS2.631, AS2.666, AS2.982, AS2.1190, AS2.1364, AS2.1396, IFFI 1001, IFFI 1002, IFFI 1005, IFFI 1006, IFFI 1008, IFFI 1009, IFFI 1010, IFFI 1012, IFFI 1021, IFFI 1027, IFFI 1037, IFFI 1042, IFFI 1043, IFFI 1045, IFFI 1048, IFFI 1049, IFFI 1050, IFFI 1052, IFFI 1059, IFFI 1060, IFFI 1063, IFFI 1202, IFFI 1203, IFFI 1206, IFFI 1209, IFFI 1210, IFFI 1211, IFFI 1212, IFFI 1213, IFFI 1215, IFFI 1220, IFFI 1221, IFFI 1224, IFFI 1247, IFFI 1248, IFFI 1251, IFFI 1270, IFFI 1277, IFFI 1287, IFFI 1289, IFFI 1290, IFFI 1291, IFFI 1292, IFFI 1293, IFFI 1297, IFFI 1300, IFFI 1301, IFFI 1302, IFFI 1307, IFFI 1308, IFFI 1309, IFFI 1310, IFFI 1311, IFFI 1331, IFFI 1335, IFFI 1336, IFFI 1337, IFFI 1338, IFFI 1339, IFFI 1340, IFFI 1345, IFFI 1348, IFFI 1396, IFFI 1397, IFFI 1399, IFFI 1411, IFFI 1413; *Saccharomyces cerevisiae* Hansen Var. ellipsoideus (Hansen) Dekker, ACCC2043, AS2.2, AS2.3, AS2.8, AS2.53, AS2.163, AS2.168, AS2.483, AS2.541, AS2.559, AS2.606, AS2.607, AS2.611, AS2.612; *Saccharomyces chevalieri* Guillermond, AS2.131, AS2.213; *Saccharomyces delbrueckii*, AS2.285; *Saccharomyces delbrueckii* Lindner var. mongolicus Lodder et van Rij, AS2.209, AS2.1157; *Saccharomyces exiguus* Hansen, AS2.349, AS2.1158; *Saccharomyces fermentati* (Saito) Lodder et van Rij, AS2.286, AS2.343; *Saccharomyces logos* van laer et

Denamur ex Jorgensen, AS2.156, AS2.327, AS2.335; *Saccharomyces mellis* Lodder et Kreger Van Rij, AS2.195; *Saccharomyces microellipsoides* Osterwalder, AS2.699; Saccharomyces *oviformis* Osterwalder, AS2.100; *Saccharomyces rosei* (Guilliermond) Lodder et kreger van Rij, AS2.287; *Saccharomyces rouxii* Boutroux, AS2.178, AS2.180, AS2.370, AS2.371; *Saccharomyces sake* Yabe, ACCC2045; *Candida arborea*, AS2.566; *Candida Krusei* (Castellani) Berkhout, AS2.1045; *Candida lambica*(Lindner et Genoud) van.Uden et Buckley, AS2.1182; *Candida lipolytica* (Harrison) Diddens et Lodder, AS2.1207, AS2.1216, AS2.1220, AS2.1379, AS2.1398, AS2.1399, AS2.1400; *Candida parapsilosis* (Ashford) Langeron et Talice, AS2.590; *Candida parapsilosis* (Ashford) et Talice Var. intermedia Van Rij et Verona, AS2.491; *Candida pulcherriman* (Lindner) Windisch, AS2.492; *Candida rugousa* (Anderson) Diddens et Loddeer, AS2.511, AS2.1367, AS2.1369, AS2.1372, AS2.1373, AS2.1377, AS2.1378, AS2.1384; *Candida tropicalis* (Castellani) Berkout, ACCC2004, ACCC2005, ACCC2006, AS2.164, AS2.402, AS2.564, AS2.565, AS2.567, AS2.568, AS2.617, AS2.1387; *Candida utilis* Henneberg Lodder et Kreger Van Rij, AS2.120, AS2.281, AS2.1180; *Crebrothecium ashbyii* (Guillermond) Routein, AS2.481, AS2.482, AS2.1197; *Geotrichum candidum* Link, ACCC2016, AS2.361, AS2.498, AS2.616, AS2.1035, AS2.1062, AS2.1080, AS2.1132, AS2.1175, AS2.1183; *Hansenula anomala* (Hansen) H et P sydow, ACCC2018, AS2.294, AS2.295, AS2.296, AS2.297, AS2.298, AS2.299, AS2.300, AS2.302, AS2.338, AS2.339, AS2.340, AS2.341, AS2.470, AS2.592, AS2.641, AS2.642, AS2.635, AS2.782, AS2.794; *Hansenula arabitolgens* Fang, AS2.887; *Hansenula jadinii* Wickerham, ACCC2019; *Hansenula saturnus* (Klocker) H et P sydow, ACCC2020; *Hansenula schneggii* (Weber) Dekker, AS2.304; *Hansenula subpelliculosa* Bedford, AS2.738, AS2.740, AS2.760, AS2.761, AS2.770, AS2.783, AS2.790, AS2.798, AS2.866; *Kloeckera apiculata* (Reess emend. Klocker) Janke, ACCC2021, ACCC2022, ACCC2023, AS2.197, AS2.496, AS2.711, AS2.714; *Lipomyces starkeyi* Lodder et van Rij, ACCC2024, AS2.1390; *Pichia farinosa* (Lindner) Hansen, ACCC2025, ACCC2026, AS2.86, AS2.87, AS2.705, AS2.803; *Pichia membranaefaciens* Hansen, ACCC2027, AS2.89, AS2.661, AS2.1039; *Rhodosporidium toruloides* Banno, ACCC2028; *Rhodotorula glutinis* (Fresenius) Harrison, ACCC2029, AS2.280, ACCC2030, AS2.102, AS2.107, AS2.278, AS2.499, AS2.694, AS2.703, AS2.704, AS2.1146; *Rhodotorula minuta* (Saito) Harrison, AS2.277; *Rhodotorula rubar* (Demme) Lodder, ACCC2031, AS2.21, AS2.22, AS2.103, AS2.105, AS2.108, AS2.140, AS2.166, AS2.167, AS2.272, AS2.279, AS2.282; *Saccharomyces carlsbergensis* Hansen, ACCC2032, ACCC2033, AS2.113, AS2.116, AS2.118, AS2.121, AS2.132, AS2.162, AS2.189, AS2.200, AS2.216, AS2.265, AS2.377, AS2.417, AS2.420,

AS2.440, AS2.441, AS2.443, AS2.444, AS2.459, AS2.595, AS2.605, AS2.638, AS2.742, AS2.745, AS2.748, AS2.1042; *Saccharomyces uvarum Beijer*, IFFI 1023, IFFI 1032, IFFI 1036, IFFI 1044, IFFI 1072, IFFI 1205, IFFI 1207; *Saccharomyces willianus* Saccardo, AS2.5, AS2.7, AS2.119, AS2.152, AS2.293, AS2.381, AS2.392, AS2.434, AS2.614, AS2.1189; *Saccharomyces sp.*, AS2.311; *Saccharomyces ludwigii* Hansen, ACCC2044, AS2.243, AS2.508; *Saccharomyces sinenses* Yue, AS2.1395; *Schizosaccharomyces octosporus* Beijerinck, ACCC 2046, AS2.1148; *Schizosaccharomyces pombe* Linder, ACCC2047, ACCC2048, AS2.248, AS2.249, AS2.255, AS2.257, AS2.259, AS2.260, AS2.274, AS2.994, AS2.1043, AS2.1149, AS2.1178, IFFI 1056; *Sporobolomyces roseus* Kluyver et van Niel, ACCC 2049, ACCC 2050, AS2.619, AS2.962, AS2.1036, ACCC2051, AS2.261, AS2.262; *Torulopsis candida* (Saito) Lodder, ACCC2052, AS2.270; *Torulopsis famta* (Harrison) Lodder et van Rij, ACCC2053, AS2.685; *Torulopsis globosa* (Olson et Hammer) Lodder et van Rij, ACCC2054, AS2.202; *Torulopsis inconspicua* Lodder et van Rij, AS2.75; *Trichosporon behrendii* Lodder et Kreger van Rij, ACCC2055, AS2.1193; *Trichosporon capitatum* Diddens et Lodder, ACCC2056, AS2.1385; *Trichosporon cutaneum*(de Beurm et al.)Ota, ACCC2057, AS2.25, AS2.570, AS2.571, AS2.1374; *Wickerhamia fluoresens* (Soneda) Soneda, ACCC2058, AS2.1388.

Certain yeast species that can be activated or induced according to the present invention and are included in the present invention are known to be pathogenic to human and/or other living organisms, for example, *Ashbya gossypii*; *Blastomyces dermatitidis*; *Candida albicans*; *Candida parakrusei*; *Candida tropicalis*; *Citeromyces matritensis*; *Crebrothecium ashbyii*; *Cryptococcus laurentii*; *Cryptococcua neoformans*; *Debaryomyces hansenii*; *Debaryomyces kloeckeri*; *Debaryomyces* sp.; *Endomycopsis fibuligera*. Under certain circumstances, it may be less preferable to use such pathogenic yeasts in the biological compositions of the invention, for example, if such use is in an open field, it may endanger the health of human and/or other living organisms.

Yeasts of the *Saccharomyces* genus are generally preferred. Among strains of *Saccharomyces cerevisiae, Saccharomyces cerevisiae* Hansen is a preferred strain. The most preferred strains of yeast are *Saccharomyces cerevisiae* strains having accession numbers AS2.504, AS2.558, AS2.413, AS2.397, AS2.69, AS2.109, AS2.607, AS2.516, AS2.561, AS2.422, AS2.393, AS2.631, AS2.982, AS2.560, AS2.467, AS2.415, AS2.375, AS2.628, AS2.1190, AS2.562, AS2.463, AS2.409, AS2.379, AS2.666, AS2.631, AS2.182, AS2.431, AS2.606, AS2.53, AS2.611, AS2.414, AS2.576, AS2.483, IFFI 1211, 1FF1 1293, IFFI 1308, IFFI 1210, IFFI 1213, IFFI 1307, IFFI 1206, IFFI 1052, IFFI 1301, IFFI 1291, IFFI 1202, IFFI 1021, IFFI 1059, IFFI 1052, IFFI 1441, IFFI 1008, IFFI 1220, IFFI 1302,

and IFFI 1023 as deposited at the China General Microbiological Culture Collection Center (CGMCC).

Generally, yeast strains useful for the invention can be obtained from private or public laboratory cultures, or publically accessible culture deposits, such as the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 and the China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China.

Although it is preferred, the preparation of the yeast cell components of the invention is not limited to starting with a pure strain of yeast. Each yeast cell component may be produced by culturing a mixture of yeast cells of different species or strains. The constituents of a yeast cell component can be determined by standard yeast identification techniques well known in the art.

The ability and efficiency of the activated yeasts to perform a desired function before or after culturing under the conditions of the invention can readily be tested by methods known in the art. For example, HPLC or mass spectrometry can be used for detecting and analyzing various organic molecules in a sample of solid waste. Microbiological methods well known in the art can be used for detecting and counting the number of viable microorganisms and the total number of microorganisms in a sample.

When treating organic manure that has a relatively high bacteria count, the biological composition can be formulated to comprise largely yeast cells that suppress bacterial growth. When the biological composition is to be used to treat solid waste that is contaminated with undesirable chemicals, the biological composition can be formulated to comprise mostly yeast cells that degrade undesirable chemicals. Thus, the biological compositions have utility in many types of situations encountered in municipal, commercial, agricultural, and industrial establishments. The invention can also be used domestically, especially in rural areas.

The biological compositions of the invention can be applied directly to the solid waste. As known to those skilled in the relevant art, many methods and appliances may be used to mix the yeasts with the solid waste. In one embodiment, a culture broth of the yeasts of the present invention are added directly to the solid waste to be treated. In another embodiment, dried powders of the yeasts of the present invention are mixed with the solid waste to which water is added at a later time. The biological compositions may be applied to and mixed with the solid waste by spreaders, sprayers, and other mechanized means which may be automated. The amount of biological composition used depends in

part on the circumstances and the type of solid waste, and can be determined empirically. However, to achieve an effective treatment, it is desired to use from about 300 to 600g dry weight (less than 10% moisture) of the biological composition per cubic meter of solid waste. The yeast cells are first mixed with water in the ratio of about 1000 g yeasts (dry weight) to about 30 liters, and then incubated for 12 to 24 hours, prior to application to the solid waste. The benefit of the treatment, e.g., reduction of odor or bacteria count, takes effect about 24 to 72 hours after the application. Although not necessary, the biological compositions of the invention can also be used in conjunction or in rotation with other types of deodorants, disinfectants, and detoxification agents.

Described respectively in Sections 5.1 - 5.4 are the yeast cell components used for degradation of antibiotics, pathogen suppression, degradation of undesirable chemicals, and reduction of odor. Methods for preparing each yeast cell components are described. Section 5.6 describes the manufacture of the biological compositions of the invention. In various embodiments of the invention, standard techniques for handling, transferring, and storing yeasts are used. Although it is not necessary, sterile conditions or clean environments are desirable when carrying out the manufacturing processes of the invention.

5.1. YEAST CELLS THAT DEGRADE ANTIBIOTICS

The present invention provides yeast cells that are capable of degrading antibiotics that are typically found in manures and sludge.

According to the invention, the ability of yeast cells to degrade antibiotics is activated or enhanced by culturing the yeast cells in the presence of an electromagnetic field in an appropriate culture medium. The resulting yeast cells can be used as a component in the biological solid waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to degrade antibiotics can generally be found in the range of 70 MHz to 600 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their enhanced ability to decompose one or more types of antibiotics by methods well known in the art. Antibiotics degraded by the yeasts of the invention include but are not limited to molecules within the families of beta-lactams, tetracyclines, polypeptides, glycopeptides, aminoglycosides, and macrolides.

The method of the invention for making antibiotics-degrading yeasts is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose,

fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(NH_4)_2HPO_4$, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

Table 1: Composition for a culture medium for yeasts that degrade antibiotics

Medium Composition	Quantity
Manure or sludge	8.0g, dry weight, >120 mesh
NaCl	0.2g
$MgSO_4 \cdot 7H_2O$	0.2g
$CaCO_3 \cdot 5H_2O$	0.5g
$CaSO_4 \cdot 2H_2O$	0.2g
Peptone	1.5g
K_2HPO_4	0.5g
Extract containing antibiotics (≥ 100 ug/ml)	600ml
Autoclaved water	400ml

The extract containing antibiotics is prepared by dispersing and incubating 500g of fresh waste, e.g., manures, sludge, in about 600ml of warm water (at 35-40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. If the extract contains only negligible amount of a particular antibiotics, an appropriate amount of the antibiotics can be added into the extract.

It should be noted that the composition of the media provided in Table 1 is not intended to be limiting. Various modifications of the culture medium may be made by

those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2-10^5 cell/ml, preferably $3x10^2$-10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 70.000 to 100.000 MHz and 410.000 to 620.000 MHz, preferably. The field strength of the EM field(s) is in the range of 40 to 250 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 144 - 384 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of 8.5-85mV/cm, usually at about 50 mV/cm, is used. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 150-250 mV, usually to about 200 mV. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m^3, preferably 0.4 mol/m^3. The oxygen level can be controlled by any

conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C. The recovered yeast cells may also be dried and stored in powder form.

To determine the activity of the activated yeast cells towards an antibiotic compound, methods well known in the art, such as HPLC, can be used to measure the amounts of the antibiotic compound in a test sample at various time point and under different incubation conditions. For example, a sample containing known concentration of an antibiotic (up to 100 mg per liter) is prepared. Then, 0.1 ml of activated and unactivated yeasts (at least 10^7 cells/ml) are added to the 100 liter samples containing the antibiotics, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the extracts are determined and compared by performing HPLC on the samples.

The method is generally applicable to many type of antibiotics. In specific embodiments, methods that are optimized for a particular type of antibiotics are described, infra.

Yeast cell component that decomposes penicillins

In a specific embodiment, a method for producing yeast cells that decompose penicillins, e.g., penicillin G and Cloxacillin, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 100.000 MHz, including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, and 100 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.399 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 77 MHz at 48 mV/cm for 15 h; 83 MHz at 48 mV/cm for 15 h; 90 MHz at 48 mV/cm for 15 h; 96 MHz at 48 mV/cm for 15 h; 77 MHz at 200 mV/cm for 30 h; 83 MHz at 200 mV/cm for 30 h; 90 MHz at 200 mV/cm for 30 h; 96 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards penicillin was determined by measuring the amounts of penicillin the activated yeast cells can degrade. Two 100 liter samples each containing a penicillin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of

antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of penicillin in the sample with the activated yeast cells was reduced by more than 56.5%.

Yeast cell component that decomposes chlortetracycline

In a specific embodiment, a method for producing yeast cells that decompose chlortetracycline, e.g., aureomycin, chlortetracycline hydrochloridum, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.748 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards chlorotetracycline was determined by measuring the amounts of chlorotetracycline the activated yeast cells can degrade. Two 100 liter samples each containing a chlorotetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the two samples. Comparing to samples with unactivated yeast cells, the amount of chlorotetracycline in the samples with the activated yeast cells was reduced by more than 62.3%.

Yeast cell component that decomposes oxytetracycline

In a specific embodiment, a method for producing yeast cells that decompose oxytetracycline, e.g., oxytetracycline hydrochloridum, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, Yeast cells of *Saccharomyces cerevisiae* strain AS2.101 are cultured at about 25-30°C in a culture

medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 74 MHz at 48 mV/cm for 15 h; 88 MHz at 44 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 74 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards oxytetracycline was determined by measuring the amounts of oxytetracycline the activated yeast cells can degrade. Two 100 liter samples each containing an oxytetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with Comparing to samples unactivated yeast cells, the amount of oxytetracycline in the samples with the activated yeast cells was reduced by more than 65.5%.

Yeast cell component that decomposes doxycycline

In a specific embodiment, a method for producing yeast cells that decompose doxycycline is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.417 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 77 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 71 MHz at 200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 77 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards doxycycline was determined by measuring the amounts of doxycycline the activated yeast cells can degrade. Two 100 liter samples each containing a doxycycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples are determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the

amount of doxycycline in the samples with the activated yeast cells was reduced by more than 54.9%.

Yeast cell component that decomposes tetracycline

In a specific embodiment, a method for producing yeast cells that decompose tetracycline is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.70 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 75 MHz at 48 mV/cm for 15 h; 82 MHz at 48 mV/cm for 15 h; 85 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 75 MHz at 200 mV/cm for 30 h; 82 MHz at 200 mV/cm for 30 h; 85 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards tetracycline was determined by measuring the amounts of tetracycline the activated yeast cells can degrade. Two 100 liter samples each containing a tetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples are determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of tetracycline in the samples with the activated yeast cells was reduced by more than 67.6%.

Yeast cell component that decomposes streptomycin

In a specific embodiment, a method for producing yeast cells that decompose streptomycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.441 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 80 MHz at 48 mV/cm for 15 h; 96 MHz at 48 mV/cm for 15 h; 70 MHz at

200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 80 MHz at 200 mV/cm for 30 h; 96 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards streptomycin was determined by measuring the amounts of streptomycin the activated yeast cells can degrade. Two 100 liter samples each containing a streptomycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of streptomycin in the samples with the activated yeast cells was reduced by more than 77.8%.

Yeast cell component that decomposes kanamycin

In a specific embodiment, a method for producing yeast cells that decompose kanamycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, east cells of *Saccharomyces cerevisiae* strain AS2.336 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 h; 78 MHz at 48 mV/cm for 15 h; 86 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 71 MHz at 200 mV/cm for 30 h; 78 MHz at 200 mV/cm for 30 h; 86 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards kanamycin was determined by measuring the amounts of kanamycin the activated yeast cells can degrade. Two 100 liter samples each containing a kanamycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of kanamycin in the samples with the activated yeast cells was reduced by more than 68.7%.

Yeast cell component that decomposes erythromycin

In a specific embodiment, a method for producing yeast cells that decompose erythromycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.422 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 73 MHz at 48 mV/cm for 15 h; 79 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 73 MHz at 200 mV/cm for 30 h; 79 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards erythromycin was determined by measuring the amounts of erythromycin the activated yeast cells can degrade. Two 100 liter samples each containing a erythromycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of erythromycin in the samples with the activated yeast cells was reduced by more than 72.7%.

Yeast cell component that decomposes spiramycin

In a specific embodiment, a method for producing yeast cells that decompose spiramycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.620 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 77 MHz at 48 mV/cm for 15 h; 84 MHz at 48 mV/cm for 15 h; 93 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 77 MHz at 200 mV/cm for 30 h; 84 MHz at 200 mV/cm for 30 h; 93 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards spiramycin was determined by measuring the amounts of spiramycin the activated yeast cells can degrade. Two 100 liter samples each containing a spiramycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of spiramycin in the samples with the activated yeast cells was reduced by more than 66.8%.

Yeast cell component that decomposes bacitracin

In a specific embodiment, a method for producing yeast cells that decompose bacitracin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.486 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 75 MHz at 48 mV/cm for 15 h; 78 MHz at 48 mV/cm for 15 h; 81 MHz at 48 mV/cm for 15 h; 95 MHz at 48 mV/cm for 15 h; 75 MHz at 200 mV/cm for 30 h; 78 MHz at 200 mV/cm for 30 h; 81 MHz at 200 mV/cm for 30 h; 95 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards bacitracin was determined by measuring the amounts of bacitracin the activated yeast cells can degrade. Two 100 liter samples each containing a bacitracin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of bacitracin in the samples with the activated yeast cells was reduced by more than 71.6%.

Yeast cell component that decomposes colistin

In a specific embodiment, a method for producing yeast cells that decompose colistin or colistin sulfate is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.611 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 433 MHz at 85 mV/cm for 12 h; 440 MHz at 85 mV/cm for 12 h; 446 MHz at 85 mV/cm for 12 h; 457 MHz at 85 mV/cm for 12 h; 433 MHz at 204 mV/cm for 24 h; 440 MHz at 204 mV/cm for 24 h; 446 MHz at 204 mV/cm for 24 h; 457 MHz at 204 mV/cm for 24 h.

The activity of the activated yeast cells towards colistin was determined by measuring the amounts of colistin the activated yeast cells can degrade. Two 100 liter samples each containing a colistin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells: After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of colistin in the samples with the activated yeast cells was reduced by more than 71.6%.

Yeast cell component that decomposes chloramphenicol

In a specific embodiment, a method for producing yeast cells that decompose chloramphenicol and salts such as chloromycetin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.371 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 419 MHz at 85 mV/cm for 12 hr; 425 MHz at 85 mV/cm for 12 h; 433 MHz at 85 mV/cm for 12 h; 462 MHz at 85 mV/cm for 12 h; 419 MHz at183 mV/cm for 24 h; 425 MHz at183 mV/cm for 24 h; 433 MHz at183 mV/cm for 24 h; 462 MHz at183 mV/cm for 24 h.

The activity of the activated yeast cells towards chloramphenicol was determined by measuring the amounts of chloramphenicol the activated yeast cells can degrade. Two 100 liter samples each containing a chloramphenicol concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of chloramphenicol in the samples with the activated yeast cells was reduced by more than 58.6%.

Yeast cell component that decomposes cephalosporins

In a specific embodiment, a method for producing yeast cells that decompose cephalosporins, e.g., cephalothin, cephaloridine, cephaloglyin, cephalolexin, and cephazoline, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.559 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 434 MHz at 85 mV/cm for 12 h, 441 MHz at 85 mV/cm for 12 h, 450 MHz at 85 mV/cm for 12 h, 458 MHz at 85 mV/cm for 12 h; 434 MHz at 198 mV/cm for 24 h, 441 MHz at 198 mV/cm for 24 h, 450 MHz at 198 mV/cm for 24 h, 458 MHz at 198 mV/cm for 24 h.

The activity of the activated yeast cells towards cephalosporins was determined by measuring the amounts of cephalothin the activated yeast cells can degrade. Two 100 liter samples each containing a cephalothin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of cephalothins in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes neomycin

In a specific embodiment, a method for producing yeast cells that decompose neomycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 550.000 to 620.000 MHz, including but not limited to 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, and 575 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.182 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 557 MHz at 85 mV/cm for 12 h, 564 MHz at 85 mV/cm for 12 h, 568 MHz at 85 mV/cm for 12 h, 574 MHz at 85 mV/cm for 12 h; 557 MHz at 231 mV/cm for 24 h, 564 MHz at 231 mV/cm for 24 h, 568 MHz at 231 mV/cm for 24 h, 574 MHz at 231 mV/cm for 24 h.

The activity of the activated yeast cells towards neomycin was determined by measuring the amounts of neomycin the activated yeast cells can degrade. Two 100 liter samples each containing a neomycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of neomycin in the samples with the activated yeast cells was reduced by more than 67.7%.

Yeast cell component that decomposes novobiocin

In a specific embodiment, a method for producing yeast cells that decompose novobiocin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 550.000 to 620.000 MHz, including but not limited to 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, and 610 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.112 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 594 MHz at 85 mV/cm for 12 h, 599 MHz at 85 mV/cm for 12 h, 602 MHz at 85 mV/cm for 12 h, 608 MHz at 85 mV/cm for 12 h; 594 MHz at 231 mV/cm for 24 h, 599 MHz at 231 mV/cm for 24 h, 602 MHz at 231 mV/cm for 24 h, 608 MHz at 231 mV/cm for 24 h.

The activity of the activated yeast cells towards novobiocin was determined by measuring the amounts of novobiocin the activated yeast cells can degrade. Two 100 liter samples each containing a novobiocin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of novobiocin in the samples with the activated yeast cells was reduced by more than 69.5%.

5.2. YEAST CELL COMPONENTS THAT DECOMPOSE UNDESIRABLE CHEMICALS

The present invention further provides yeast cells that are capable of degrading chemicals that are typically found in solid waste.

According to the invention, the ability of yeast cells to degrade undesirable chemicals is activated or enhanced by culturing the yeast cells in the presence of an electromagnetic field in an appropriate culture medium. The resulting yeast cells can be used as a component in the biological waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to degrade undesirable chemicals can generally be found in the range of 30 to 280 MHz, 410 to 440 MHz, 660 to 690 MHz, 1400 to 1435 MHz, and 1980 to 2210 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their enhanced ability to decompose one or more types of chemicals by methods well known in the art. Undesirable chemicals degraded by the yeasts of the invention include but are not limited to herbicides, pesticides, and fertilizer-related pollutants.

The method of the invention for making chemical-degrading yeasts is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5%

and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(NH_4)_2HPO_4$, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

Table 2: Composition for a culture medium for yeasts that degrade chemicals

Medium Composition	Quantity
Manure or sludge	8.0g, dry weight, >120 mesh
NaCl	0.2g
$MgSO_4 \cdot 7H_2O$	0.2g
$CaCO_3 \cdot 5H_2O$	0.5g
$CaSO_4 \cdot 2H_2O$	0.2g
Peptone	1.5g
K_2HPO_4	0.5g
Extract containing chemicals (≥ 100 ug/ml)	600ml
Autoclaved water	400ml

The extract for the culture medium is prepared by incubating 500g of fresh waste, e.g., manures, sludge, and/or garbage, in about 600ml of warm water (at 35-40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. If the extract contains low amount of the particular chemicals, an appropriate amount of the chemical can be added into the extract.

It should be noted that the composition of the media provided in Table 2 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2-10^5 cell/ml, preferably $3x10^2$-10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series

of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the ranges of 30.000 to 100.000, 70.000 to 280.000, 410.000 to 430.000, 660.000 to 680.000 and 1980.000 to 2210.000 MHz. The field strength of the EM field(s) is in the range of 40 to 250 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 90 - 480 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of about 8 to about 300 mV/cm is used. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m^3, preferably 0.4 mol/m^3. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C. The recovered yeast cells may also be dried and stored in powder.

To determine the activity of the activated yeast cells towards a chemical compound, methods well known in the art, such as HPLC, can be used to measure the amounts of the compound in a test sample at various time point and under different incubation conditions. For example, a sample containing a known concentration of a chemical compound (up to 100 mg per liter) is prepared. Then, 0.1 ml of activated and

unactivated yeasts (at least 10^7 cells/ml) were added to the 100 liter samples containing the compound, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of compound remaining in the extracts are determined and compared by performing HPLC on the samples.

The method is generally applicable to many classes of chemicals. In specific embodiments, method that are optimized for a particular class of chemicals are described, infra.

Yeast cell component that decomposes aromatic compounds

In specific embodiment, method for producing yeast cells that decompose trichlorophenol, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 30.000 to 100.000 MHz, or preferably 52 to 98 MHz including but not limited to 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98 MHz. Yeast cells of *Saccharomyces cerevisiae* strain IFFI1411 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 6 EM fields in the order stated: 82 MHz at 82 mv/cm for 25 h; 90 MHz at 82 mv/cm for 25 h; 98 MHz at 82mv/cm for 25 h; 82 MHz at 274 mv/cm for 32 h; 90 MHz at 274 mv/cm for 32 h; 98 MHz at 274 mv/cm for 25 h.

The activity of the activated yeast cells towards trichlorophenol was determined by measuring the amounts of the compound the activated yeast cells can degrade. Two 100 liter samples each containing the compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of the compound remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of tripchlorophenol in the samples with the activated yeast cells was reduced by more than 56.4%.

In another specific embodiment, method for producing yeast cells that decompose toluene or ethylbenzene, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 52.000 to 98.000 MHz, including but not limited to 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.56 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a

series of 4 EM fields in the order stated: 76 MHz at 89 mV/cm for 20 h; 80 MHz at 89 mV/cm for 200 h; 86 MHz at 89 mV/cm for 20 h; and 96 MHz at 89 mV/cm for 20 h.

The activity of the activated yeast cells towards toluene or ethylbenzene was determined by measuring the amounts of the compounds the activated yeast cells can degrade. Two 100 liter samples each containing the compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of the compound remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of toluene in the samples with the activated yeast cells was reduced by more than 74.3%.

In another specific embodiment of the invention, a method for producing yeast cells that decompose dimethylbenzene compounds, e.g., p-xylene, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 30.000 to 50.000 MHz, or 70.000 to 98.000 MHz including but not limited to 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.420 are cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 72 MHz at 93 mV/cm for 20 h; 80 MHz at 93 mV/cm for 20 h; 88 MHz at 93 mV/cm for 20 h; and 98 MHz at 93 mV/cm for 20 h.

The activity of the activated yeast cells towards dimethylbezene compounds was determined by measuring the amounts of dimethylbezene compounds the activated yeast cells can degrade. Two 100 liter samples each containing a dimethylbenzene compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of p-xylene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of p-xylene in the samples with the activated yeast cells was reduced by more than 66.6%.

Yeast cell component that decomposes aldehyde compounds

In another specific embodiment of the invention, a method for producing yeast cells that decompose benzaldehyde and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 133-151 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, or 151 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.374 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 130 mV/cm for 30 h; 86 MHz at 130 mV/cm for 30 h; 94 MHz at 130 mV/cm for 30 h; 96 MHz at 130 mV/cm for 30 h.

The activity of the activated yeast cells towards benzaldehyde was determined by measuring the amounts of benzaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing benzaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of benzaldehyde remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of benzaldehyde in the samples with the activated yeast cells was reduced by more than 63.6%.

In yet another specific embodiment, a method for producing yeast cells that decompose propylaldehyde and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 145-162 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, and 162 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.414 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 103 mV/cm for 20 h; 88 MHz at 103 mV/cm for 20 h; 96 MHz at 103 mV/cm for 20 h; 98 MHz at 103 mV/cm for 30 h.

The activity of the activated yeast cells towards propylaldehyde was determined by measuring the amounts of propylaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing propylaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast

cells. After 24 hours, the amounts of propylaldehyde remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of propylaldehyde in the samples with the activated yeast cells was reduced by more than 73.8%.

In yet another specific embodiment, a method for producing yeast cells that decompose nenanthaldehyde compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 MHz or 100.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.503 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 81 MHz at 90 mV/cm for 12 h, 85 MHz at 90 mV/cm for 12 h, 89 MHz at 90 mV/cm for 12 h, 94 MHz at 90 mV/cm for 12 h, 81 MHz at 157 mV/cm for 24 h, 85 MHz at 157 mV/cm for 24 h, 89 MHz at 157 mV/cm for 24 h, 94 MHz at 157 mV/cm for 24 h.

The activity of the activated yeast cells towards nenanthaldehyde was determined by measuring the amounts of nenanthaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing nenanthaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of nenanthaldehyde remaining in the samples were determined and compared by performing HPLC on the two samples. Comparing to samples with unactivated yeast cells, the amount of nenanthaldehyde in the samples with the activated yeast cells was reduced by more than 81.3% in 24 hours.

<u>Yeast cell component that decomposes halogen-substituted benzene compounds</u>

In a specific embodiment, method for producing yeast cells that decompose halogen-substituted benzene compounds, e.g., m-dichlorobenzene, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 163.000 to 183.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178. 179, 180, 181, 182, and 183 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.483 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order

stated: 72 MHz at 107 mV/cm for 20 h; 80 MHz at 107 mV/cm for 10 h; 90 MHz at 107 mV/cm for 30 h; 94 MHz at 107 mV/cm for 40 h.

The activity of the activated yeast cells towards dichlorobenzene was determined by measuring the amounts of dichlorobenzene the activated yeast cells can degrade. Two 100 liter samples each containing dichlorobenzene concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dichlorobenzene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dichlorobenzene in the samples with the activated yeast cells was reduced by more than 64.6%.

Yeast cell component that decomposes acetophenone and related compounds.

In yet another embodiment, a method for producing yeast cells that decompose acetophenone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 175.000 to 191.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 175. 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, and 191 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.265 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 124 mV/cm for 20 h; 82 MHz at 124 mV/cm for 30 h; 90 MHz at 124 mV/cm for 40 h; 98 MHz at 124 mV/cm for 20 h.

The activity of the activated yeast cells towards acetophenone was determined by measuring the amounts of acetophenone the activated yeast cells can degrade. Two 100 liter samples each containing acetophenone at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of acetophenone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of acetophenone compounds in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes arsanilic acid and related compounds

In yet another embodiment, a method for producing yeast cells that decompose arsanilic acid and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 183.000 to 205.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, and 205 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.745 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 133 mV/cm for 30 h; 88 MHz at 133 mV/cm for 40 h; 92 MHz at 133 mV/cm for 30 h; 96MHz at 133 mV/cm for 30 h.

The activity of the activated yeast cells towards arsanilic acid was determined by measuring the amounts of arsanilic acid compounds the activated yeast cells can degrade. Two 100 liter samples each containing an arsanilic acid at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of arsanilic acid antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of arsanilic acid in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes roxarsone and related compounds

In another specific embodiment, a method for producing yeast cells that decompose roxarsone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 114.000 to 128.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, and 128 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.173 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 110 mV/cm for 10 h; 92 MHz at 110 mV/cm for 10h; 78 MHz at 213 mV/cm for 30 h; 92 MHz at 213 mV/cm for 30 h.

The activity of the activated yeast cells towards roxarsone was determined by measuring the amounts of roxarsone the activated yeast cells can degrade. Two 100 liter samples each containing roxarsone concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of roxarsone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of roxarsone in the samples with the activated yeast cells was reduced by more than 67.9%.

Yeast cell component that decomposes furazolidonum compounds

In yet another specific embodiment, a method for producing yeast cells that decompose furazolidonum and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 200.000 to 220.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, and 220 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.397 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 74 MHz at 98 mV/cm for 30 h; 76 MHz at 98 mV/cm for 20 h; 86 MHz at 98 mV/cm for 30 h; 94 MHz at 98 mV/cm for 30 h.

The activity of the activated yeast cells towards furazolidonum was determined by measuring the amounts of furazolidonum the activated yeast cells can degrade. Two 100 liter samples each containing furazolidonum concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of furazolidonum remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of furazolidonum in the samples with the activated yeast cells was reduced by more than 81.4%.

Yeast cell component that decomposes Decoquinate

In yet another specific embodiment, a method for producing yeast cells that decompose decoquinate and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 213.000 to 229.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, and 229 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.452 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 112 mV/cm for 30 h; 82 MHz at 112 mV/cm for 30 h; 86 MHz at 112 mV/cm for 30 h; 94 MHz at 112 mV/cm for 20 h.

The activity of the activated yeast cells towards decoquinate was determined by measuring the amounts of decoquinate the activated yeast cells can degrade. Two 100 liter samples each containing decoquinate concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of decoquinate remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of decoquinate in the samples with the activated yeast cells was reduced by more than 67.9%.

Yeast cell component that decomposes Trichlorophonum compounds

In yet another specific embodiment, a method for producing yeast cells that decompose trichlorophonum and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 220.000 to 250.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, and 250 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.100 were cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 74 MHz at 219 mV/cm for 30 h; 86 MHz at 219 mV/cm for 20 h; 96 MHz at 219 mV/cm for 30 h; 98 MHz at 219 mV/cm for 20 h.

The activity of the activated yeast cells towards trichlorophonum was determined by measuring the amounts of trichlorophonum the activated yeast cells can degrade. Two 100 liter samples each containing trichlorophonum concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated

yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of trichlorophonum remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of trichlorophonum in the samples with the activated yeast cells was reduced by more than 72.4%.

Yeast cell component that decomposes Dinitolmide

In a specific embodiment, method for producing yeast cells that decompose dinitolmide and related compounds is provided. Dinitolmide is 2-methyl-3,5-dinitrobenzamide and is also known as zoalene. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 220.000 to 250.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, and 250 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.189 are cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 202 mV/cm for 30 h; 82 MHz at 202 mV/cm for 30 h; 90 MHz at 202 mV/cm for 20 h; 96 MHz at 202 mV/cm for 20 h.

The activity of the activated yeast cells towards dinitolmide was determined by measuring the amounts of zoalene compounds the activated yeast cells can degrade. Two 100 liter samples each containing a dinitolmide concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dinitolmide remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dinitolmide in the samples with the activated yeast cells was reduced by more than 72.4%.

Yeast cell component that removes ammonium compound (NH_4)

In a specific embodiment, a method for producing yeast cells that remove or reduce the level of ammonium compounds in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 660 to 680 MHz or 2160

to 2190 MHz, and preferably 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.614 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 662 MHz at 152 mv/cm for 18 h; 666 MHz at 152 mv/cm for 18 h; 672 MHz at 152 mv/cm for 18 h; 678 MHz at 152 mv/cm for 18 h; 662 MHz at 310 mv/cm for 25 h; 666 MHz at 310 mv/cm for 25 h; 672 MHz at 310 mv/cm for 35 h; 678 MHz at 310 mv/cm for 35 h.

The activity of the activated yeast cells was determined by measuring the amounts of ammonium compounds removed by the activated yeast cells. The amount of ammonium compounds in the samples with the activated yeast cells was reduced significantly (>93.6%) compared to the sample containing unactivated yeast cells.

Yeast cell component that removes nitrates and nitrites

In a specific embodiment, a method for producing yeast cells that remove or reduce the level of nitrates and nitrites in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 661.000 to 680.000 MHz including but not limited to 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, and 680 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.14 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 661 MHz at 126 mV/cm for 25 h; 665 MHz at 126 mV/cm for 25 h; 672 MHz at 126 mV/cm for 25 h; 676 MHz at 126 mV/cm for 25 h; 661 MHz at 196 mV/cm for 25 h; 665 MHz at 196 mV/cm for 25 h; 672 MHz at 196 mV/cm for 38 h; 676 MHz at 196 mV/cm for 38 h.

The activity of the activated yeast cells towards nitrates was determined by measuring the amounts of nitrates removed by the activated yeast cells. Two 100 liter samples each containing nitrates at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of nitrates remaining in the samples were determined and compared by performing HPLC

on the samples. Comparing to samples with unactivated yeast cells, the amount of nitrates in the samples with the activated yeast cells was reduced by more than 69.7%

Yeast cell component that remove biologically available phosphorus

In a specific embodiment, a method for producing yeast cells that remove biologically available phosphorus, e.g., HPO_4^{2-}, $H_2PO_4^-$, etc., is provided. In a specific embodiment, a method for producing yeast cells that remove or reduce the level of nitrates and nitrites in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 80.000 to 440.000 MHz, preferably 86.000 to 120.000 MHz or 410.000 to 440.000 MHz including but not limited to 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, and 430 MHz. For example, Yeast cells of *Saccharomyces cerevisiae* strain AS2.620 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 98 MHz at 68 mv/cm for 24 h; 112 MHz at 68 mv/cm for 24 h; 108 MHz at 68 mv/cm for 24 h; 118 MHz at 68 mv/cm for 24 h; 98 MHz at 240 mv/cm for 24 h; 112 MHz at 240 mv/cm for 24 h; 108 MHz at 240 mv/cm for 42 h; 118 MHz at 240 mv/cm for 42 h.

The activity of the activated yeast cells towards available phosphorus was determined by measuring the amounts of available phosphorus the activated yeast cells can remove. Two 100 liter samples each containing available phosphorus concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of phosphorous remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of available phosphorus in the samples with the activated yeast cells was reduced by more than 65.8%.

Yeast cell component that decomposes Trichlorphon

In a specific embodiment, a method for producing yeast cells that decompose trichlorphon and related organophosphate pesticide compounds is provided. The

frequencies of the EM field(s) used to activate the yeast cells are in the range of 1980.000 to 2020.000, and preferably 2000.000 to 2020.000 including but not limited to 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, and 2020 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.440 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 2000 MHz at 125mv/cm for 10 h; 2004 MHz at 125 mv/cm for 10 h; 2009 MHz at 125 mv/cm for 24 h; 2018 MHz at 125 mv/cm for 24 h; 2000 MHz at 168 mv/cm for 10 h; 2004 MHz at 168mv/cm for 10 h; 2009 MHz at 168 mv/cm for 56 h; 2018 MHz at 168 mv/cm for 56 h.

The activity of the activated yeast cells towards trichlorphon was determined by measuring the amounts of trichlorphon the activated yeast cells can degrade. Two 100 liter samples each containing trichlorphon concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of trichlorphon remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of trichlorphon in the samples with the activated yeast cells was reduced by more than 10% in 48 hours.

Yeast cell component that decomposes Dichlorvos

In a specific embodiment, a method for producing yeast cells that decompose dichlorvos (DDVP) and related organophosphate pesticide compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.443 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1993 MHz at 140 mV/cm for 24 h; 2023 MHz at 140 mV/cm for 24 h; 2083 MHz at 140 mV/cm for 24 h; 2103 MHz at 140 mV/cm for 24 h; 1993 MHz at 190 mV/cm for 24 h; 2023 MHz at 190 mV/cm for 24 h; 2083 MHz at 190 mV/cm for 56 h; 2103 MHz at 190 mV/cm for 56 h.

The activity of the activated yeast cells towards dichlorvos was determined by measuring the amounts of dichlorvos the activated yeast cells can degrade. Two 100 liter

samples each containing dichlorvos concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of dichlorvos remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dichlorvos in the samples with the activated yeast cells was reduced by more than 67.5%.

Yeast cell component that decomposes Momocrotophos

In a specific embodiment, a method for producing yeast cells that decompose momocrotophos and related insecticides is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.93 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 2998 MHz at 165 mV/cm for 24 h; 2033 MHz at 165 mV/cm for 24 h; 2058 MHz at 165 mV/cm for 24 h; 2113 MHz at 165 mV/cm for 24 h; 2998 MHz at 202 mV/cm for 56 h; 2033 MHz at 202 mV/cm for 56 h; 2058 MHz at 202 mV/cm for 24 h; 2113 MHz at 202 mV/cm for 24 h.

The activity of the activated yeast cells towards momocrotophos was determined by measuring the amount of momocrotophos the activated yeast cells can degrade. Two 100 liter samples each containing momocrotophos concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of monocrotophos remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of momocrotophos in the samples with the activated yeast cells was reduced by more than 73.4%.

Yeast cell component that decomposes Dimethoate

In a specific embodiment, a method for producing yeast cells that decompose dimethoate and related insecticidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.379 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1988 MHz at 195 mV/cm for 24 h; 2023 MHz at 195 mV/cm for 24 h; 2088 MHz at 195 mV/cm for 24 h; 2108 MHz at 195 mV/cm for 24 h; 1988 MHz at 277 mV/cm for 56 h; 2023 MHz at 277 mV/cm for 56 h; 2088 MHz at 277 mV/cm for 24 h; 2108 MHz at 277 mV/cm for 24 h.

The activity of the activated yeast cells towards dimethoate was determined by measuring the amount of dimethoate the activated yeast cells can degrade. Two 100 liter samples each containing dimethoate concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dimethoate remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dimethoate in the samples with the activated yeast cells was reduced by more than 69.6%.

Yeast cell component that decomposes DDT

In a specific embodiment, a method for producing yeast cells that decompose DDT and related dilorinated organic insecticidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1420.000 to 1435.000 including but not limited to 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.415 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1423 MHz at 75 mV/cm for 24 h; 1426 MHz at 75 mV/cm for 24 h; 1433 MHz at 75 mV/cm for 24 h; 1435 MHz at 75 mV/cm for 24 h; 1423 MHz at 146 mV/cm for 56 h; 1426 MHz at 146 mV/cm for 56 h; 1433 MHz at 146 mV/cm for 24 h; 1435 MHz at 146 mV/cm for 24 h.

The activity of the activated yeast cells towards DDT was determined by measuring the amount of DDT the activated yeast cells can degrade. Two 100 liter samples each containing DDT concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of DDT remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of DDT in the samples with the activated yeast cells was reduced by more than 78.5%.

Yeast cell component that decomposes Toxaphene

In a specific embodiment, a method for producing yeast cells that decompose toxaphene and related chlorinated organic insecicidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1420.000 to 1435.000 including but not limited to 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.504 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 1420 MHz at 120 mV/cm for 24 h; 1426 MHz at 120 mV/cm for 24 h; 1431 MHz at 120 mV/cm for 24 h; 1434 MHz at 120 mV/cm for 24 h.

The activity of the activated yeast cells towards toxaphene was determined by measuring the amount of toxaphene the activated yeast cells can degrade. Two 100 liter samples each containing toxaphene concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of toxaphene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of toxaphene in the samples with the activated yeast cells was reduced by more than 70.8%.

5.3. ODOR-REDUCING YEAST CELL COMPONENTS

The present invention also provides yeast cells that are capable of reducing the odor of solid waste, e.g., manures, sludge, and/or garbage. Without being bound by any theory, the inventor believes that the yeast cells of the invention are capable of reducing the

odor of solid waste by modifying, assimilating, or decomposing known and unknown compounds in the solid waste that are malodorous. However, it is not necessary to demonstrate that such compounds have been decomposed. It is sufficient so long as the odor is reduced as determined subjectively by a panel of subjects, after the yeast cells of the invention have been used.

According to the present invention, yeast cells that are capable of reducing the odor of solid waste are prepared by culturing the cells in the presence of an electromagnetic field in an appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing this ability in yeasts can generally be found in the range of 2160 to 2380 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their ability to reduce the odor of solid waste by methods well known in the art.

The method of the invention for making the odor-reducing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(NH_4)_2HPO_4$, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

It should be noted that the composition of the media provided in Table 38 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2-10^5 cell/ml, preferably $3x10^2$-10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the

same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 2160 to 2380 MHz, and preferably in the ranges of 2160.000 to 2250.000 MHz or 2280.000 to 2380.000 MHz. The field strength of the EM field(s) is in the range of 25 to 300 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 80-320 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of 25-200 mV/cm. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 250-300mV/cm. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m^3, preferably 0.4 mol/m^3. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0-4°C. The recovered yeast cells may also be dried and stored in powder form.

Any methods known in the art can be used to test the cultured yeast cells for their ability to reduce the odor of organic materials. The amount of malodorous chemicals such as hydrogen sulfide, ammonia, indole, p-cresol, skatol, and organic acids present in a test sample of organic material can be determined by any methods known in the art,

including but not limited to gas phase chromatography, olfactometry, mass spectrometry, or the use of an odor panel.

For example, to determine the activity of the activated yeast cells towards an malodorous compound, mass spectrometry (e.g., VG micromass) can be used to measure the amounts of the malodorous compound in a test sample at various time point and under different incubation conditions. For example, a known amount of a malodorus compound (up to 100 mg per liter) is added to 10 liter of an aqueous extract of manure. Then, 0.1 ml of activated and unactivated yeasts (at least 10^7 cells/ml) are added to the 10 liter samples containing the compound, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of the malodorous compounds remaining in the extracts are determined and compared.

Yeast cell component that reduce odor caused by sulfur containing compounds

In one embodiment of the invention, a method for producing yeast cells that remove hydrogen sulfide and other related sulfur-containing or sulfhydryl (SH-) containing molecules is provided. Yeast cells that remove hydrogen sulfide and other related sulfur-containing or sulfhydryl (SH-) containing molecules can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.559 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2165 MHz at 240 mV/cm for 20 hr; 2175 MHz at 240 mV/cm for 20-60 hr; 2200 MHz at 240 mV/cm for 20 hr; 2235 MHz at 240 mV/cm for 20 hr.

The activity of the activated yeast cells towards sulfur-containing or sulfhydryl (SH-) compounds was determined by measuring the change in amount of hydrogen sulfides in the presence of the activated yeast cells. Two 100 liter samples each containing hydrogen sulfide concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of hydrogen sulfide remaining in the samples were determined and compared. Comparing to

samples with unactivated yeast cells, the amount of hydrogen sulfide in the samples with the activated yeast cells was reduced by more than 59.8%.

Yeast cell component that reduce odor caused by NH-containing containing compounds

In another embodiment of the invention, a method for producing yeast cells that remove ammonia and related NH-containing compounds is provided. Yeast cells that remove ammonia and related NH-containing compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.423 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2160 MHz at 250 mV/cm for 20 hr; 2175 MHz at 250 mV/cm for 20 hr; 2210 MHz at 250 mV/cm for 20 hr; 2245 MHz at 250 mV/cm for 10 hr.

The activity of the activated yeast cells towards ammonia acid NH-containing compounds was determined by measuring the change in the amount of ammonia in the presence of the activated yeast cells. Two 100 liter samples each containing NH-containing compounds at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of ammonia remaining in the samples were determined and compared. Comparing to samples with unactivated yeast cells, the amount of ammonia in the samples with the activated yeast cells was reduced by more than 69.6%.

Yeast cell component that reduce odor caused by indole and other related compounds

In the invention, a method for producing yeast cells that decompose indole and other related compounds, such as skatol is provided. Yeast cells that decompose indole and other related compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.612 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2165 MHz at 240 mV/cm for 40 hr; 2180 MHz at 240 mV/cm for 20 hr; 2200 MHz at 240 mV/cm for 40 hr; 2220 MHz at 240 mV/cm for 20 hr.

The activity of the activated yeast cells towards indole and other related compounds was determined by measuring the amount of indole removed by the activated yeast cells. Two 100 liter samples each containing indole related compounds at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of indole remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of indole in the samples with the activated yeast cells was reduced by more than 71.3%.

Yeast cell component that reduce odor caused by organic acids

In yet another embodiment of the invention, a method for producing yeast cells that remove odorous organic acids, e.g., formic acid, acetic acid, propanoic acid, butyric acid, and other volatile fatty acids, is provided. Yeast cells that can reduce the odor of organic acids can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.53 are cultured at about 25-30°C in a culture medium as described in Table 42 in the presence of a series of 4 EM fields in the order stated: 2315 MHz at 290 mV/cm for 30 hr; 2335 MHz at 290 mV/cm for 10 hr; 2355 MHz at 290 mV/cm for 20 hr; 2375 MHz at 290 mV/cm for 10 hr.

The activity of the activated yeast cells towards organic acids was determined by measuring the change in the amounts of acetic acid in the presence of the activated yeast cells. Two 100 liter samples each containing organic acids concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and

incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of acetic acid remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of acetic acid in the samples with the activated yeast cells was reduced by more than 89.4%.

Yeast cell component that reduce odor caused by aliphatic substituted amine

In yet another embodiment of the invention, a method for producing yeast cells that remove or degrade aliphatic substituted amine, such as methylamine, dimethylamine, or trimethylamine thereby reducing the odor caused by such compounds, is provided. Yeast cells that remove or degrade such amines can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.541 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2160 MHz at 250 mV/cm for 20 hr; 2190 MHz at 250 mV/cm for 10 hr; 2210 MHz at 250 mV/cm for 40 hr; 2250 MHz at 250 mV/cm for 40 hr.

The activity of the activated yeast cells towards methyl-substituted amine was determined by measuring the amount of such amine in the presence of the activated yeast cells. Two 100 liter samples each containing methyl-substituted amine at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of methyl-substituted amines remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of methyl-substituted amines in the samples with the activated yeast cells was reduced by more than 82.2%.

Yeast cell component that reduce odor caused by p-cresol and related compounds

In yet another embodiment of the invention, a method for producing yeast cells that remove or degrade p-cresol and related compounds is provided. Yeast cells that

remove or degrade p-cresol and other related compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.163 were cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2300 MHz at 98 mV/cm for 20 hr; 2370 MHz at 98 mV/cm for 15 hr; 2300 MHz at 250 mV/cm for 20 hr; 2370 MHz at 250 mV/cm for 30 hr.

The activity of the activated yeast cells towards p-cresol and related compounds was determined by measuring the change in the amounts of p-cresol and related compounds in the presence of the activated yeast cells. Two 100 liter samples each containing p-cresol at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of p-cresol remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of p-cresol in the samples with the activated yeast cells was reduced by more than 92.5%.

5.4. PATHOGEN-SUPPRESSING YEAST CELL COMPONENTS

The present invention also provides yeast cells that are capable of suppressing the proliferation of pathogenic microorganisms that are present in solid waste. Typically, due to an abundance of nutrients present in solid waste for such pathogenic microorganisms, the numbers of pathogens increase rapidly over a period of time. However, in the presence of the pathogen-suppressing yeasts of the invention, the numbers of pathogens in the treated solid waste remains unchanged, or decreases over time. Without being bound by any theory or mechanism, the inventor believes that the presence of the pathogen-suppressing yeasts in the solid waste creates an environment that is unfavorable for the growth of pathogenic microorganisms.

According to the invention, the ability of yeasts to affect/control the numbers of pathogens is activated or enhanced by culturing the yeasts in the presence of an electromagnetic field. The resulting pathogen-suppressing yeast cells are used as a component in the solid waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to control the numbers of pathogenic microorganisms can generally be

found in the range of 30 MHz to 50 MHz. After sufficient time is given for the yeast cells to grow, the cells can be tested for their ability to affect/control the number of pathogens by methods well known in the art.

The method of the invention for making pathogen-suppressing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(NH_4)_2HPO_4$, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

Table 4: Composition for a culture medium for Pathogen-Suppressing yeasts

Medium Composition	Quantity
Soluble Starch	8.0g
Sucrose	5g
NaCl	0.2g
$MgSO_4 \cdot 7H_2O$	0.2g
$CaCO_3 \cdot 5H_2O$	0.5g
$CaSO_4 \cdot 2H_2O$	0.2g
Peptone	1.5g
K_2HPO_4	0.5g
Autoclaved water	400ml
Pathogen extract	600ml

The pathogen extract for the culture medium is prepared by incubating 500g of pathogen-containing waste in about 600ml of warm water (at 35°C to 40°C) for 24 hours

at 30-37°C, and filtering the fluid to remove particulate matters. It should be noted that the composition of the media provided in Table 4 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2-10^5 cell/ml, preferably $3x10^2$-10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 30.000 to 50.000, 500.000 to 650.000, and 1000.000 to 1150.000 MHz. The field strength of the EM field(s) is in the range of 20 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 144 - 272 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of 20-180 mV/cm. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 200-350 mV/cm.

At the end of the culturing process, the pathogen-suppressing yeast cells may be recovered from the culture by various methods known in the art, and stored at about 0°C to 4°C. The pathogen-suppressing yeast cells may also be dried and stored in powder form.

The ability of the pathogen-suppressing yeasts to control the numbers of pathogens can be determined by any methods known in the art for enumerating microorganisms, such as optical density, plating out dilutions on solid media for counting, or counting individual cells under a microscope. Stains may be applied to distinguish or identify different strains or species of microorganisms present in a sample, or to determine their viability. When a range of pathogenic microorganisms are expected to be affected by the pathogen-suppressing yeasts, the numbers of more than one representative species of pathogenic microorganisms can be monitored to assess the performance of the pathogen-suppressing yeasts.

For example, samples of solid waste containing a known concentration of pathogenic microorganisms are cultured under the same conditions for a same period of time in the presence of different concentrations of pathogen-suppressing yeasts, and as negative control, the same strain of yeasts that have not been treated according to the culturing methods of the invention. A sample without any added yeast may also be included to determine the growth of pathogens under normal circumstances. The numbers of pathogens before and after the culture period are determined and compared.

A one liter culture containing at least 10^{10} cells of a pathogenic microorganism per ml is prepared. One ml of activated yeast cells (containing 2 to 5 x 10^7 yeasts per ml) is added to the one liter culture of pathogenic microorganism and incubated at 30°C for 24 hours. A control is included which contained unactivated yeast cells. The numbers of microorganisms in the respective culture is then determined and compared. The following are several examples of which a particular species of pathogenic bacteria was studied.

Yeast cell component that suppresses *Staphylococcus aureus*

In a specific embodiment of the invention embodiment, a method for producing yeast cells that suppress the growth of *Staphylococcus aureus* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.595 were cultured at about 25-30°C in a culture medium as described

in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12h; 43 MHz at 26 mV/cm for 12 h; 47 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 43 MHz at 150 mV/cm for 24 h; 47 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *Staphylococcus aureus* was determined by measuring the growth of *Staphylococcus aureus* in the presence of the activated yeast cells. *Staphylococcus aureus* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Staphylococcus aureus* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.6%.

Yeast cell component that suppresses *Diplococcus pneumoniae*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *Diplococcus pneumoniae* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain IFFI1021 were cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12h; 42 MHz at 26 mV/cm for 12 h; 49 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 42 MHz at 150 mV/cm for 24 h; 49 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *Diplococcus pneumoniae* was determined by measuring the growth of *Diplococcus pneumoniae* in the presence of the activated yeast cells. *Diplococcus pneumoniae* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Diplococcus pneumoniae* in the samples were determined by conventional

bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 3 %.

Yeast cell component that suppresses *Bacillus anthracis*

In a specific embodiment, a method for producing yeast cells that suppress *the growth of Bacillus anthracis* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 20.000 to 45.000 MHz, including but not limited to 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, and 45 MHz. For example, yeast cells of *Bacillus anthracis* strain AS2.390 are cultured at about 25-30 °C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 24 MHz at 100 mv/cm for 24 h, 37 MHz at 100 mv/cm for 24 h, 40 MHz at 100 mv/cm for 24 h, 45 MHz at 100 mv/cm for 24 h, 24 MHz at 190 mv/cm for 24 h, 37 MHz at 190 mv/cm for 24 h, 40 MHz at 190 mv/cm for 24 h, 45 MHz at 190 mv/cm for 24 h.

The activity of the activated yeast cells towards *Bacillus anthracis* was determined by measuring the growth of *Bacillus anthracis* in the presence of the activated yeast cells. *Bacillus anthracis* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Bacillus anthracis* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.6 %.

Yeast cell component that suppresses *Mycobacterium tuberculosis*

In a specific embodiment, a method for producing yeast cells that suppress *Mycobacterium tuberculosis* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.431 are cultured at about 25-30 ° in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 33 MHz at 26 mv/cm for 12 h; 36 MHz at 26 mv/cm for 12 h; 45 MHz

at 26 mv/cm for 12 h; 47 MHz at 26 mv/cm for 12 h; 33 MHz at 150 mv/cm for 24 h; 36 MHz at 150 mv/cm for 24 h; 45 MHz at 150 mv/cm for 24 h; 47 MHz at 150 mv/cm for 24 h.

The activity of the activated yeast cells towards *Mycobacterium tuberculosis* was determined by measuring the growth of *Mycobacterium tuberculosis* in the presence of the activated yeast cells. *Mycobacterium tuberculosis* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Mycobacterium tuberculosis* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.9 %.

Yeast cell component that suppresses *E. coli*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *E. coli* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.561 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 34 MHz at 26 mV/cm for 12 h; 38 MHz at 26 mV/cm for 12 h; 49 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 34 43 MHz at 150 mV/cm for 24 h; 38 MHz at 150 mV/cm for 24 h; 49 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *E. coli* was determined by measuring the growth of *E. coli* in the presence of the activated yeast cells. *E. coli* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *E. coli* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with

unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 48%.

Yeast cell component that suppresses *Salmonella*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *Salmonella* species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.178 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 33 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12 h; 38 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 33 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 38 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *Salmonella* species was determined by measuring the growth of *Salmonella* in the presence of the activated yeast cells. *Salmonella* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Salmonella* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 62 %.

Yeast cell component that suppresses Vibrio species

In a specific embodiment, a method for producing yeast cells that suppress Vibrio species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 500.000 to 550.000 MHz, including but not limited to 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, and 540 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.377 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 521 MHz at 150 mv/cm for 24 h, 527 MHz at 150

mv/cm for 24 h, 531 MHz at 150 mv/cm for 24 h, 538 MHz at 150 mv/cm for 24 h, 521 MHz at 276 mv/cm for 24 h, 527 MHz at 276 mv/cm for 24 h, 531 MHz at 276 mv/cm for 24 h, 538 MHz at 276 mv/cm for 24 h.

The activity of the activated yeast cells towards *Vibrio* species was determined by measuring the growth of *Vibrio* species in the presence of the activated yeast cells. *Vibrio* species contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Vibrio* species in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 5.6 %.

Yeast cell component that suppresses Shigella species

In a specific embodiment, a method for producing yeast cells that suppress Shigella species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 600.000 to 650.000 MHz, including but not limited to 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, and 650 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.395 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 630 MHz at 180 mv/cm for 24 h, 636 MHz at 180 mv/cm for 24 h, 641 MHz at 180 mv/cm for 24 h, 649 MHz at 180 mv/cm for 24 h, 630 MHz at 314 mv/cm for 24 h, 636 MHz at 314 mv/cm for 24 h, 641 MHz at 314 mv/cm for 24 h, 649 MHz at 314 mv/cm for 24 h.

The activity of the activated yeast cells towards *Shigella* species was determined by measuring the growth of *Shigella* species in the presence of the activated yeast cells. *Shigella* species contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Shigella* species in the samples were determined by conventional bacteria cell counting

method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 4.6 %.

Yeast cell component that suppresses *Clostridium botulinum*

In a specific embodiment, a method for producing yeast cells that suppress *Clostridium botulinum* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1000.000 to 1050.000 MHz, including but not limited to 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, and 1035 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.432 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 1012 MHz at 180 mv/cm for 24 h, 1018 MHz at 180 mv/cm for 24 h, 1024 MHz at 180 mv/cm for 24 h, 1033 MHz at 180 mv/cm for 24 h, 1012 MHz at 323 mv/cm for 24 h, 1018 MHz at 323 mv/cm for 24 h, 1024 MHz at 323 mv/cm for 24 h, 1033 MHz at 323 mv/cm for 24 h.

The activity of the activated yeast cells towards *Clostridium botulinum* was determined by measuring the growth of *Clostridium botulinum* in the presence of the activated yeast cells. *Clostridium botulinum* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Clostridium botulinum* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 5.1 %.

Yeast cell component that suppresses *Bacillus aerogenes capsulatus*

In a specific embodiment, a method for producing yeast cells that suppress *Bacillus aerogenes capsulatus.* The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1100.000 to1150.000 MHz, including but not limited to 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, and 1120 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.432 are cultured at about 25-30°C in a culture medium as described in

Table 4 in the presence of a series of 8 EM fields in the order stated: 1102 MHz at 180 mv/cm for 24 h, 1106 MHz at 180 mv/cm for 24 h, 1113 MHz at 180 mv/cm for 24 h, 1117 MHz at 180 mv/cm for 24 h, 1102 MHz at 301 mv/cm for 24 h, 1106 MHz at 301 mv/cm for 24 h, 1113 MHz at 301 mv/cm for 24 h, 1117 MHz at 301 mv/cm for 24 h.

The activity of the activated yeast cells towards *Bacillus aerogenes capsulatus* was determined by measuring the growth of *Bacillus aerogenes capsulatus* in the presence of the activated yeast cells. *Bacillus aerogenes capsulatus* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Bacillus aerogenes capsulatus* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 6.2 %.

5.5 ADAPTATION

In another embodiment of the invention, activated yeast cells prepared according to any one of Sections 5.1-5.10 can be further cultured as a mixture in the presence of a sample of the solid waste which is to be treated. This optional process which improves the performance of the solid waste treatment compositions is described by way of an example as follows.

An extract of the solid waste to be treated, such as manure or sludge, is prepared by mixing and soaking about 1000 g of poultry manure in 1000 to 3000 ml of water for about 48 hours. The extract is then mixed with about 1000 g of dried manure (dry weight, i.e., less than 10% moisture) to form a suspension to which the yeast cells are added. At least 1 ml of yeasts which contains more than $5x10^7$ cell/ml is added to the suspension. Depending on the number of strains of activated yeast cells used, up to about 50 ml of yeast cells can be added. If only a few strains are used, 5 to 10ml of yeast cells per strain can be added. The process can be scaled up or down according to needs. The mixture of yeast and solid waste is cultured for about 120-280 hours in the presence of a series of electromagnetic fields. Each electromagnetic field has a frequency that, depending on the strains of yeast included, corresponds to one of the frequencies described in Sections 5.1-5.4. If many different strains of yeasts are used, a combination of the following five frequency bands can be used : 20-50 MHz, 60-150 MHz, 400-700 MHz, 1400-1600 MHz,

2000-2500 MHz; each for about 24 to 56 hours. Generally, the yeast cells are subjected to an EM field strength in the range from 20mV/cm to 350mV/cm in this process.

The culture is incubated at temperatures that cycle between about 5°C to about 37°C. For example, in a typical cycle, the temperature of the culture may start at about 37°C and be kept at this temperature for about 1-2 hours, then adjusted to 26-30°C and kept at this temperature for about 2-4 hours, and then brought down to 5 -10°C and kept at this temperature for about 1-2 hours, and then the temperature may be raised again to about 37°C for another cycle. The cycles are repeated until the process is completed. After the last temperature cycle is completed, the temperature of the culture is lowered to 3-4°C and kept at this temperature for about 5-6 hours. After the process, the yeast cells may be isolated and recovered from the medium by conventional methods, such as filtration. The adapted yeast cells can be stored under 4°C. An exemplary set-up of the culture process is depicted in Figure 2.

5.6 MANUFACTURE OF THE BIOLOGICAL COMPOSITIONS

The biological composition of the present invention can be produced by culturing yeast cells under appropriate conditions according to Section 5.1 to 5.4, and mixing the desired amounts of cultures of yeast cells. Since the biological composition is not immediately used to treat solid waste, the yeasts of the biological composition can be dried in a two-stage drying process. During the first drying stage, the yeast cells are dried in a first dryer at a temperature not exceeding 65°C for a period of time not exceeding 10 minutes so that yeast cells quickly become dormant. The yeast cells are then sent to a second dryer and dried at a temperature not exceeding 70°C for a period of time not exceeding 30 minutes to further remove water. After the two stages, the water content should be lower than 5%. It is preferred that the temperatures and drying times be adhered to in both drying stages so that yeast cells do not lose their vitality and functions. The dried yeast cells are then cooled to room temperature. The dried yeast cells may also be screened in a separator so that particles of a preferred size are selected. The dried cells can then be sent to a bulk bag filler for packing.

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of

the invention. Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for treatment of solid waste comprising antibiotics, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the antibiotics, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 100 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of penicillin, chlortetracycline, oxytetracycline, doxycycline, tetracycline, streptomycin, kanamycin, erythromycin, spiramycin and bacitracin;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 410 to 470 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of colistin, chloramphenicol, and cephalothin; or

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 550 to 620 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of neomycin and novobiocin.

2. A method for treatment of solid waste comprising undesirable chemicals , said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following::

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 52 to 98 MHz and a field strength of 8 to 300 mV/cm, which can degrade toluene, ethylbenzene, or trichlorophenol;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 30 to 50 MHz or 70 to 98 MHz and a field strength of 8 to 250 mV/cm; and which can degrade dimethylbenezene compounds;

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 133 to 151 MHz and a field strength of 8 to 250 mV/cm, and which can degrade benzaldehyde;

(d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 145 to 162 MHz and a field strength of 8 to 250 mV/cm, and which can degrade propylaldehyde;

(e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 100 MHz and a field strength of 8 to 250 mV/cm, and which can degrade nenanthaldehyde; and

(f) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 163 to 183 MHz and a field strength of 8 to 250 mV/cm, and which can degrade dichlorobenzene.

3. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 175 to 191 MHz and a field strength of 8 to 250 mV/cm, and which can degrade acetophenone;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 183 to 205 MHz and a field strength of 8 to 250 mV/cm, and which can degrade arsanilic acid;

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 114 to 128 MHz and a field strength of 8 to 250 mV/cm, and which can degrade is roxarsone;

(d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of

70 to 98 MHz or 200 to 220 MHz and a field strength of 8 to 250 mV/cm and which can degrade furazolidonum;

(e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 213 to 229 MHz and a field strength of 8 to 250 mV/cm and which can degrade decoquinate; and

(f) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 220 to 250 MHz and a field strength of 8 to 250 mV/cm, and which can degrade trichlorophonum or dinitomide.

4. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 660 to 680 MHz or 2160 to 2190 MHz and a field strength of 25 to 300 mV/cm, and which can reduce the amount of ammonium compounds;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 661 to 680 MHz and a field strength of 8 to 250 mV/cm, and which can reduce the amount of nitrites or nitrates; or

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 86 to 120 MHz or 410 to 430 MHz and a field strength of 8 to 250 mV/cm, and which can reduce the amount of phosphates.

5. A method for treatment of solid waste comprising undesirable chemicals , said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1980 to 2118 MHz and a field strength of 25 to 300mV/cm, and which can

degrade undesirable chemical selected from the group consisting of trichlorphon, dichlorvos, momocrotophos and dimethoate;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1420 to 1435 MHz and a field strength of 25 to 300mV/cm, and which can degrade DDT or toxaphene.

6. A method for reducing the odor of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of odorous molecules in the solid waste, wherein said yeast are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 2160 to 2380 MHz and a field strength of 25 to 300 mV/cm, and said odorous molecules are selected from the group consisting of hydrogen sulfide, ammonia, indole, skatol, acetic acid, methylamine, and p-cresol.

7. A method for treatment of solid waste comprising pathogenic bacteria, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to suppress the growth of pathogenic bacteria in the solid waste, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 20 to 50 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of Staphylococcus aureus, Diplococcus pneumoniae, Bacillus anthracis, Mycobacterium tuberculosis, Salmonella species, or E. coli;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 500 to 550 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of Vibrio species;

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 600 to 650 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of Shigella species;

(d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of

1000 to 1050 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of Clostridium botulinum; and

(e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1100 to 1150 MHz and a field strength of 20 to 50 mV/cm, and which can suppress the growth of Bacillus aerogenes capsulatus.

8. A method for treatment of solid waste comprising adding a biological composition to the solid waste, said biological composition comprising at least one of the following yeast cell components:

(a) a first yeast cell component comprising a plurality of yeast cells that degrade antibiotics in solid waste, said first yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 70 to 100 MHz, 410 to 470 MHz, and 550 to 620 MHz and a field strength of 8 to 250 mV/cm;

(b) a second yeast cell component comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste, said second yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 30 to 100 MHz, 70 to 280 MHz, 410 to 430 MHz, 660 to 680 MHz and 1980 to 2210 MHz and a field strength of 8 to 250 mV/cm;

(c) a third yeast cell component comprising a plurality of yeast cells that reduce the amount of odorous molecules in solid waste, said third yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 2160 to 2380 MHz and a field strength of 25 to 300 mV/cm;

(d) a fourth yeast cell component comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in the solid waste, said fourth yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 30 to 50 MHz, 500 to 550 MHz, 600 to 650 MHz, 1000 to 1050 MHz, and 1100 to 1150 MHz and a field strength of 20 to 350 mV/cm; and

allowing the yeast cells in the yeast cell component(s) to reduce the amount of antibiotics, undesirable chemicals, odorous compounds and pathogenic bacteria in the solid waste.

9. The method of claim 8, wherein the biological composition comprises the yeast cell components of (a), (b), (c) and (d).

10. The method of claim 8 wherein said yeast cells are cells of a species of yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces delbrueckii*, *Saccharomyces exiguus*, *Saccharomyces fermentati*, *Saccharomyces logos*, *Saccharomyces mellis*, *Saccharomyces microellipsoides*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum Beijer*, *Saccharomyces willianus*, *Saccharomyces ludwigii*, *Saccharomyces sinenses*, and *Saccharomyces carlsbergensis*.

11. The method of claim 8 wherein said yeast cells are *Saccharomyces cerevisiae* cells.

12. The method of claim 11 wherein said biological composition comprises dried yeast cells, and about 300 to 600g of the biological composition is added per cubic meter of solid waste.

13. The method of claim 11 wherein prior to adding said dried yeast cells to said solid waste, said dried yeast cells are mixed with water in the ratio of about 1000 g yeast cells to about 30 liters, and incubated for about 12 to 24 hours.

14. A composition comprising a plurality of yeast cells that degrade antibiotics in solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 70 to 100 MHz, 410 to 470 MHz, and 550 to 620 MHz and (ii) a field strength of 8 to 250 mV/cm.

15. A composition comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 30 to 100 MHz, 70 to 280 MHz, 410 to 430 MHz, 660 to 680 MHz and 1980 to 2210 MHz and (ii) a field strength of 8 to 250 mV/cm.

16. A composition comprising a plurality of yeast cells that reduce the odor of solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range of 2160 to 2380 MHz and (ii) a field strength of 25 to 300 mV/cm;

17. A composition comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 30 to 50 MHz, 500 to 550 MHz, 600 to 650 MHz, 1000 to 1050 MHz, and 1100 to 1150 MHz and (ii) a field strength of 20 to 350 mV/cm.

18. The composition of claim 14, 15, 16, or 17, wherein said yeast cells are cells of a species of yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces delbrueckii*, *Saccharomyces exiguus*, *Saccharomyces fermentati*, *Saccharomyces logos*, *Saccharomyces mellis*, *Saccharomyces microellipsoides*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum Beijer*, *Saccharomyces willianus*, *Saccharomyces ludwigii*, *Saccharomyces sinenses*, and *Saccharomyces carlsbergensis*.

19. The composition of claim 14, 15, 16, or 17, wherein said yeast cells are dried yeast cells.

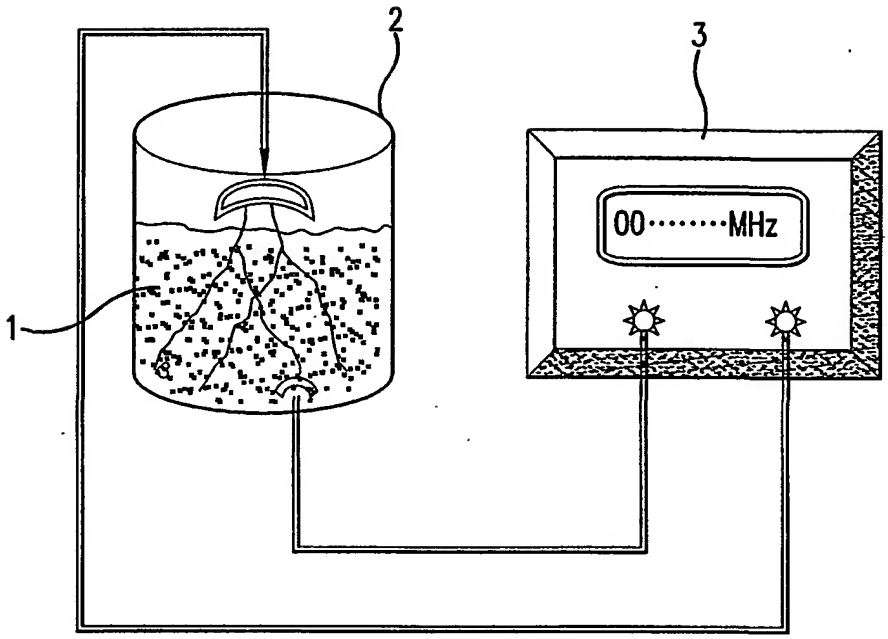
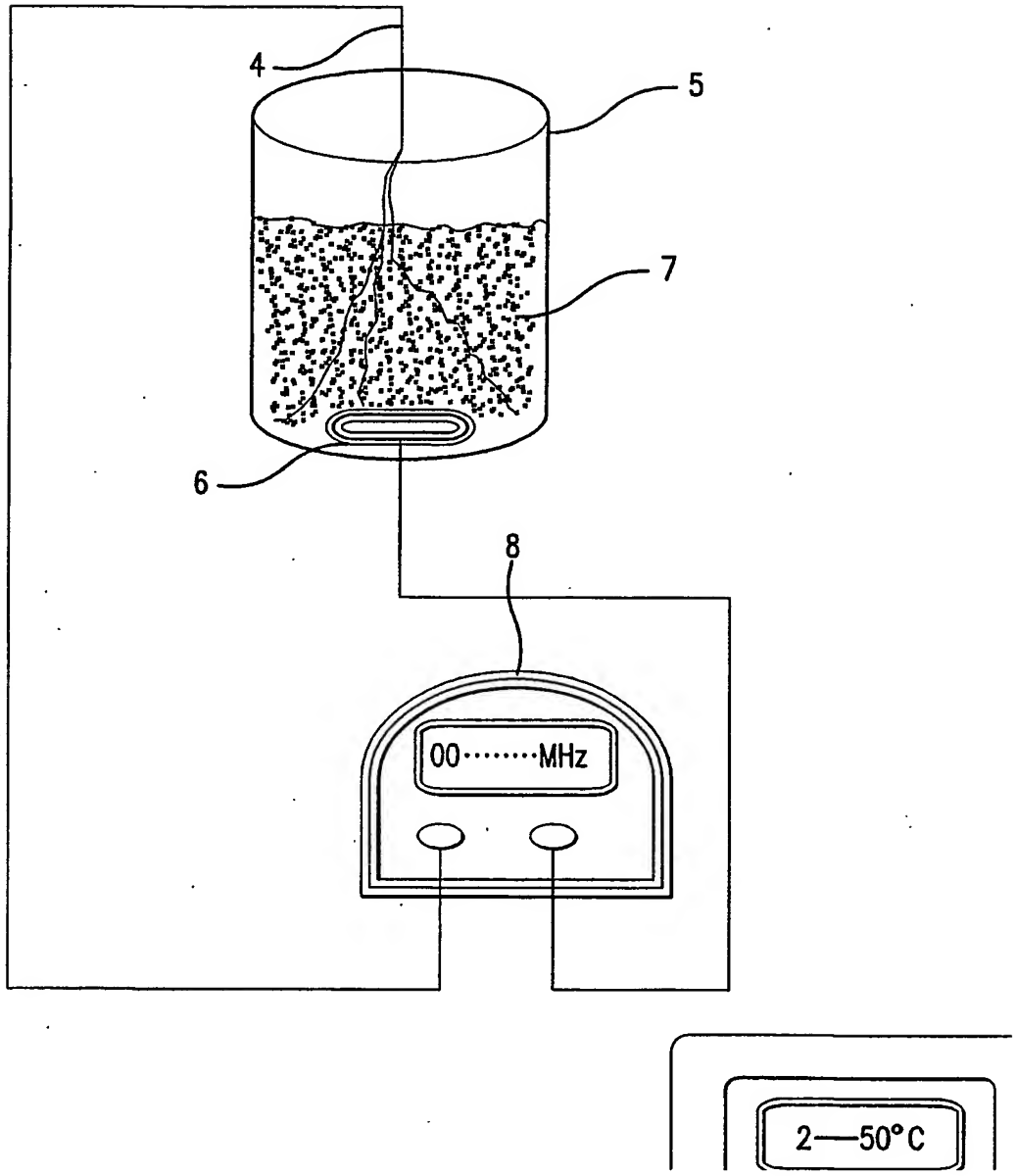


FIG.1

2/2



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 September 2002 (12.09.2002)

PCT

(10) International Publication Number
WO 2002/070683 A3

(51) International Patent Classification[7]: C12N 13/00, 1/16, 1/04, C12P 1/02, A62D 3/00, B09B 3/00 // (C12P 1/02, C12R 1:645)

(21) International Application Number:
PCT/GB2002/000915

(22) International Filing Date: 1 March 2002 (01.03.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

09/797,371	1 March 2001 (01.03.2001)	US
09/797,372	1 March 2001 (01.03.2001)	US
09/797,377	1 March 2001 (01.03.2001)	US
09/797,378	1 March 2001 (01.03.2001)	US
09/797,381	1 March 2001 (01.03.2001)	US
09/797,382	1 March 2001 (01.03.2001)	US
09/797,437	1 March 2001 (01.03.2001)	US
09/797,493	1 March 2001 (01.03.2001)	US

(71) Applicant *(for all designated States except US)*: **ULTRA BIOTECH LIMITED** [GB/GB]; 60 Circular Road, 2nd Floor, Douglas IM1 1SA (GB).

(72) Inventor; and

(75) Inventor/Applicant *(for US only)*: **CHEUNG, Ling, Yuk** [CN/CN]; 2 Dai Fu Street, Tai Po Industrial Estate, New Territories, Hong Kong (CN).

(74) Agents: **McCALLUM, William, Potter** et al.; Cruikshank & Fairweather, 19 Royal Exchange Square, Glasgow G1 3AE (GB).

(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

(88) Date of publication of the international search report:
20 February 2003

(48) Date of publication of this corrected version:
5 February 2004

(15) Information about Correction:
see PCT Gazette No. 06/2004 of 5 February 2004, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: BIOLOGICAL COMPOSITIONS FOR SOLID WASTE TREATMENT

(57) Abstract: The present invention relates to biological compositions useful for the treatment of solid waste. The biological compositions of the invention comprises a plurality of yeast cells which is capable of suppression of growth of pathogenic microorganisms, breakdown of undesirable chemicals, such as antibiotics, insecticides and waste chemicals, and reducing the odor of organic waste matters. The yeast cells of the invention are produced by culturing the plurality of yeast cells under activation conditions in the presence of a series of electromagnetic fields. The invention also relates to methods for manufacturing the treatment composition.

WO 2002/070683 A3

BIOLOGICAL COMPOSITIONS FOR SOLID WASTE TREATMENT

1. FIELD OF THE INVENTION

The invention relates to biological compositions that comprise yeasts for the treatment of solid waste. The yeasts in the compositions of the invention have been stimulated to perform a variety of functions including degradation of chemicals, reduction of odor and suppression of microorganisms. The invention also relates to methods for manufacturing the biological compositions, and methods for using the biological compositions to treat waste.

2. BACKGROUND OF THE INVENTION

Large amounts of solid waste are generated daily by industrial and agricultural activities, and by municipalities. If the waste is not treated properly, it can cause severe and long-lasting damage to the environment. In 1995-1996, 208 million tons of municipal solid waste were generated in the United States. Of the municipal solid waste generated, 56 million tons (27 percent) were recovered by recycling or composting, 33.5 million tons (16 percent) were combusted at high temperatures, and 118.5 million tons (57 percent) were landfilled.

Municipal waste may be treated instead of landfilled. One type of municipal waste treatment involves high temperature burning of the waste in an incinerator. This combustion of municipal waste significantly reduces its volume. The ash from municipal waste combustion must be properly managed to prevent the environmental damage from any potential hazardous constituents. Also, emissions from the incinerator's smoke stack must be within acceptable regulatory levels.

In the agricultural area, despite the importance of mineral fertilizers in providing mankind with abundant agricultural products, the harm done to the environment has been recognized in recent years. Mineral fertilizers may incurred damages to soils. For example, most nitrogen fertilizers may acidify soils, thereby adversely affecting the growth of plants and other soil organisms. Extensive use of chemical nitrogen fertilizers may also inhibit the activity of natural nitrogen fixing microorganisms, thereby decreasing the natural fertility of soils. The long term use of mineral fertilizers may also cause severe environmental pollution. For example, the loss of nitrogen and phosphate fertilizers due to leaching and soil erosion has led to contamination of soil and ground water, and eutrophication of surface water.

Another type of agricultural waste is manure which, if not stored or disposed of properly, can pose health and environmental threats. For example, it can cause air pollution, i.e., odor and dust; and contamination of surface and ground water with excess nutrients, organic matter, salts, and pathogens. For example, manure contains pathogenic microorganisms, such as *Escherichia coli, Salmonella spp.,* and *Shigella spp.*

Overall, cleaning up pollution as a result of a poor waste management strategy has been a complicated and difficult task. The cost for such a task is also astronomical. Thus, there is a need for inexpensive and effective methods to handle the waste generated by the myriad of human activities.

The use of biological compositions in pollution control has been proposed for many situations. Biological fertilizers utilizing microorganisms have been proposed as alternatives to mineral fertilizers. Naturally occurring nitrogen fixing microorganisms including bacteria, such as *Rhizobium, Azotobacter,* and *Azospirillum,* (See for example, U. S. Patent No. 5,071,462) and fungi, such as *Aspergillus flavus-oryzae,* (See, for example, U. S. Patent No. 4,670,037) have been utilized in biological fertilizers. Naturally occurring microorganisms capable of solubilizing phosphate rock ore or other insoluble phosphates into soluble phosphates have also been utilized in biological fertilizers either separately (e.g., U. S. Patent No. 5,912,398) or in combination with nitrogen fixing microorganisms (e.g., U. S. Patent No. 5,484,464). An approach based on recombinant DNA techniques has been developed to create more effective nitrogen fixing, phosphorus decomposing, and potassium decomposing bacterial strains for use in a biological fertilizer, see, for example, U.S. Patent No. 5,578,486; PCT publication WO 95/09814; Chinese patent publication: CN 1081662A; CN 1082016A; CN 1082017A; CN 1103060A; and CN 1109595A.

Citation of documents herein is not intended as an admission that any of the documents cited herein is pertinent prior art, or an admission that the cited documents are considered material to the patentability of the claims of the present application. All statements as to the date or representations as to the contents of these documents are based on the information available to the applicant and does not constitute any admission as to the correctness of the dates or contents of these documents.

3. SUMMARY OF THE INVENTION

The present invention relates to biological compositions useful for the treatment of solid waste. The biological compositions of the invention comprises a plurality of yeast cells which is capable of suppression of growth of pathogenic microorganisms,

breakdown of undesirable chemicals, such as antibiotics, insecticides and waste chemicals, and reducing the odor of organic waste matters.

In various embodiments, the present invention uses yeasts that are commercially available and/or accessible to the public, such as but not limited to *Saccharomyces cerevisiae*. The yeast cells of the invention are produced by culturing the plurality of yeast cells under activation conditions in the presence of a series of electromagnetic fields, such that the yeast cells become highly efficient in performing certain metabolic functions. Accordingly, the invention also relates to methods for manufacturing the treatment composition comprising culturing the yeast cells under activation conditions, mixing various yeast cell cultures of the present invention, followed by drying the yeast cells and packing the final product.

In particular, the invention encompasses methods for treatment of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the antibiotics in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The antibiotics that can be degraded by the yeast cells of invention include are not limited to penicillin, chlortetracycline, oxytetracycline, doxycycline, tetracycline, streptomycin, kanamycin, erythromycin, spiramycin, bacitracin, colistin, chloramphenicol, cephalothin, neomycin and novobiocin.

The invention also encompass methods for treatment of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The undesirable chemicals that can be degraded by the yeast cells of the invention include but are not limited to toluene, ethylbenzene, trichlorophenols, dimethylbenezenes, benzaldehyde, propylaldehyde, nenanthaldehyde, dichlorobenzenes, acetophenone, arsanilic acid, roxarsone, furazolidonum, decoquinate, trichlorophonum, dinitomide, dichlorvos, momocrotophos, dimethoate, DDT and toxaphene. The undesirable chemicals also include organic and inorganic salts such as ammonium compounds, nitrites or nitrates, and phosphates.

The invention further encompasses methods for reducing the odor of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of odorous molecules in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of

electromagnetic fields having specific ranges of frequencies and field strengths. The odorous molecules include but are not limited to hydrogen sulfide, ammonia, indole, skatol, acetic acid, methylamine, and p-cresol.

The invention further encompasses methods for suppressing the growth of pathogenic bacteria in solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to suppress the growth of pathogenic bacteria in the solid waste, wherein said yeast cells are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having specific ranges of frequencies and field strengths. The pathogenic bacteria are selected from the group consisting of *Staphylococcus aureus, Diplococcus pneumoniae, Bacillus anthracis, Mycobacterium tuberculosis, Salmonella* species, *E. coli, Vibrio* species, *Shigella* species, *Clostridium botulinum*, and *Bacillus aerogenes capsulatus*.

The methods of the invention can be carried out using combinations of the yeast cells in the treatment of solid waste. A biological composition of the invention is added to the solid waste, said biological composition comprising at least one of the following yeast cell components (a) a first yeast cell component comprising a plurality of yeast cells that degrade antibiotics in solid waste; (b) a second yeast cell component comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste; (c) a third yeast cell component comprising a plurality of yeast cells that reduce the amount of odorous molecules in solid waste; and (d) a fourth yeast cell component comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in the solid waste. The amount of time for the treatment can be determined empirically by monitoring the change in levels of the antibiotics, undesirable chemicals, pathogenic bacteria, and malodorous molecules in the solid waste, and can range from several hours, several days, and up to two or more weeks.

The invention further include methods for using the biological compositions of the present invention for the management, storage, processing, recycling or disposal of solid waste.

4. BRIEF DESCRIPTION OF FIGURES

Fig. 1 Activation of yeast cells. 1 yeast cell culture; 2 container; 3 electromagnetic field source.

Fig. 2. Adaptation of yeast cells to a soil type. 4 input electrode; 5 container; 6 electrode; 7 yeast cell culture; 8 electromagnetic field source; 9 temperature controller.

5. DETAILED DESCRIPTION OF THE INVENTION

The present invention provides biological compositions that comprise yeast cells. The present invention also provides methods for manufacturing the biological compositions as well as methods for using the biological compositions.

The biological compositions of the invention are useful for the treatment of solid waste so as to reduce the health risk and its impact on the environment that are often associated with its storage, transportation, processing, recycling and/or disposal. The use of such compositions may lower the overall cost of managing solid waste for a community, a business, or a farm, and make feasible the recycling of certain types of solid waste. As used herein, treatment of a solid waste refers to a process which changes the physical, chemical, or biological character(s) of the solid waste that make the solid waste less objectionable while it is being stored, transported, recycled, handled, or less of a health threat or an environmental threat, than if the solid waste is not treated. The treatment generally renders the waste less hazardous, or makes the solid waste safer to transport, store, handle or recycle.

According to the invention, the biological compositions comprise a plurality of yeast cell components. Each yeast cell component is a population of yeast cells which comprises a plurality of yeast cells that are capable of performing one or more desired functions falling within the following categories: (1) suppression of growth of pathogens, (2) degradation of undesirable chemicals, or (3) reducing the odor of organic materials.

In one embodiment, a biological composition of the invention comprises at least one yeast cell component that can perform a function in one of the three categories. In preferred embodiments, the biological compositions of the invention comprises yeast cell components that provide functions in all three categories. Thus, the preferred biological fertilizer compositions comprise at least three different yeast cell components. It will be understood that different alternative formulations of yeast cell components are contemplated.

As used herein, the term "solid waste" broadly refers to any kind of material that is discarded because it has served its purpose or it is a by-product that is of no use, and that includes physiological waste excreted by humans and animals. The sources of solid

waste include residential, commercial, agricultural and industrial activities. Non-industrial and non-agricultural solid waste such as trash or garbage collected from urban areas, contains discarded food materials or materials used in food preparation, and other assorted dry materials, such as paper, fabric, or plastics. Especially in residential areas and commercial areas which have restaurants and hotels, the predominant type of solid waste, herein referred to as "garbage", comprises mainly decomposable food wastes. Garbage which supports growth of pathogenic organisms and becomes malodorous due to decay, can be efficiently treated by the biological compositions of the invention. The type of solid waste that lends itself to treatment by the biological compositions of the invention has the characteristic of a high organic content.

Another type of solid waste that can be treated by the compositions of the invention is sludge. The term "sludge" as used herein broadly encompasses any solid matter that has settled out of suspension in the course of sewage storage and/or treatment, for example but not limited to, residues in a waste lagoon, residues in an urban sewage treatment plant, or sewage concentrate. The term "sludge" also include semi-solid matters, and mixtures of effluent and sediments. The term thus encompasses sludge having a wide range of viscosity, density, and water content, as well as sludge which has been partially processed or stabilized. Depending on the source, sludge may contain a variety of undesirable chemicals that may have an adverse impact on the environment if it is not properly treated. Sludge is malodorous, and supports growth of pathogenic organisms.

The biological compositions of the invention can also treat waste products produced as a result of agricultural activities. Typically, the waste is produced by animals in operations such as but not limited to ranches, farms, slaughterhouses, and markets. The continuous production and accumulation of large amounts of animal excrement creates a malodorous environment, and poses a health risk to humans and livestock due to the presence of pathogenic microorganisms. Agricultural waste can also contain undesirable chemicals, such as antibiotic feed additives, chemical fertilizers, pesticides, and herbicides, that may pollute the environment if the waste is not managed properly.

The term "animal manure" as used herein broadly encompasses organic material that comprises the feces and urine of feedlot animals with or without accompanying litter such as straw, hay, or bedding, that is traditionally used to fertilize land. Poultry manure includes but are not limited to manure produced by domesticated birds, such as chicken, duck, turkey, goose, quail, squab, ostrich, and the like. Poultry manure include excrement or guano produced by non-domesticated bird species. Cattle manure as used herein encompasses waste from domesticated ruminant mammals, such as dairy cows, or

beef cattle. The term "cattle manure" as used herein is not limited to just cattle but include other animals that graze, and that are kept primarily for their milk, meat, skin, hair, and pelts. Cattle manure includes but are not limited to manure produced by buffalos, bisons, yaks, horses, donkeys, mules, sheep, goats, camels, and the like. Cattle manure also include excrement produced by non-domesticated herds. The term "swine manure" as used herein includes but are not limited to manure produced by swines, hogs, pigs, and the like. Other agricultural waste include field crop residues, bagasse, waste from fruit and vegetable packing facilities, waste from animal product packing facilities which include animal carcasses.

In various embodiments, the biological compositions of the invention are particularly useful in treating garbage, sludge, and manure.

Municipal waste in many cases is temporarily stored at waste transfer stations. At the transfer station, waste is off-loaded from local collection routes and in some cases sorted according to type. The waste is then loaded onto larger trucks or rail cars for transport to either a municipal waste treatment or disposal facility. Generally, depending on the source of the solid waste, glass, metal, wood, and other inorganic or non-decomposable items are separated from waste of a high organic content through sorting and separating operations. These can be carried out by methods well known in the recycling / garbage disposal industry, such as mechanically, using differences in such physical characteristics of the solid waste as size, and density. Shredding or pulverizing can reduce the size of the waste articles to fine particles, resulting in a uniform mass of material which can be more easily handled, e.g., mixing or transport. Due to the variation of constituents in manure, sludge, or garbage, it may be desirable to subject a sample of a batch of waste material to analysis to determine the amount and type of pathogenic organisms and undesirable chemicals present in the batch.

While the following terms are believed to have well-defined meanings in the art, the following are set forth to facilitate explanation of the invention.

As used herein, the phrase "suppressing the growth of pathogens" refers to a decrease or lack of increase in the number of pathogenic microorganisms present in a sample of solid waste over a period of time, as a result of the presence of the yeast cells of the invention in the sample. It is to be understood that in the absence of the yeast cells, the number of pathogens in the sample would increase naturally. Many such microorganisms cause diseases in humans and animals, and may include bacteria such as *Escherichia* species, *Salmonella* species, *Shigella* species, *Mycobacterium* species, *Staphylococcus* species, *Bacillus* species, *Streptococcus* species, and *Diplococcus* species.

As used herein, the phrase "degradation of undesirable chemicals" refers to biological or biochemical processes which result in the conversion of chemical compounds that are undesirable, e.g., environmental toxins, in solid waste to an inactive form, such as the breakdown of such compounds into lower molecular weight compounds. Antibiotics are commonly present in manure and such compounds are not desired in a fertilizer made from manure because of the potential risk of ingestion by humans, for example, by eating vegetables grown using a fertilizer comprising contaminated organic material, and the possible spread of antibiotic resistance in the environment. Many antibiotics are added to animal feed to protect various farm animals, such as chicken, turkey, and swine, from bacterial and parasitic diseases, and to promote growth. A significant amount of antibiotic feed additive is excreted by the animals, and thus accumulates in manure and sludge. Many kinds of antibiotics have been used in animal operations, such as but not limited to aminoglycosides, tetracyclines, beta-lactams, glycopeptides, and macrolides. Examples of antibiotics approved for use in farms in United States include but are not limited to, bacitracin methylene disalicylate, bacitracin zinc, bambermycins, oxytetracycline, chlortetracycline, penicillin, tylosin/sulfamethazine, roxarsone, nitrasone, monensin, lasalocid, carbodox, tiamulin, hygromycin B, nystatin, novobiocin, sulfadimethoxine, ormetroprim, lincomycin, fenbendazole, and virginiamycin. The presence and quantity of such antibiotics in a composition can be determined by any methods known in the art, for example, high performance liquid chromatography (HPLC).

As used herein, the phrase "reducing the odor of organic materials" refers to a process which results in a lower concentration of one or more odorous compounds in the organic materials present in solid waste. Odorous compounds, such as but not limited to hydrogen sulfide, ammonia, indole, skatole (i.e, 3-methyl-1H-indole), p-cresol, and organic acids, are known to contribute to the malodorous quality of solid waste. The concentration of such malodorous compounds in, for example, poultry manure or in a sample of air in contact with the manure can be determined by any method well known in the art, including but not limited to gas chromatography, or mass spectrometry. Odor is a perception of smell by an organism with olfactory organs. A reduction of the intensity of the odor associated with solid waste can be determined subjectively. Various methods and techniques are known to measure the intensity of an odor. One subjective measurement of odor intensity is to measure the dilution necessary so that the odor is imperceptible or doubtful to a human or animal test panel. Alternatively, a recognition threshold may also be used which is a higher concentration at which the character of the odor is recognized. Any methods and

techniques for objectively or subjectively determine the intensity of an odor can be used to monitor the performance of the compositions and methods of the invention.

The inventor discovered that, under certain culture conditions, yeasts can be activated to become highly efficient in certain metabolic functions which result in an ability of the activated yeasts to suppress growth of pathogens, degrade undesirable chemicals, or reduce the odor of organic materials.

According to the invention, a yeast cell component of the biological fertilizer composition is produced by culturing a plurality of yeast cells in an appropriate culture medium in the presence of an alternating electromagnetic field or multiple alternating electromagnetic fields in series over a period of time. The culturing process allows yeast spores to germinate, yeast cells to grow and divide, and can be performed as a batch process or a continuous process. As used herein, the terms "alternating electromagnetic field", "electromagentic field" or "EM field" are synonymous. An electromagnetic field useful in the invention can be generated by various means well known in the art. A schematic illustration of exemplary setups are depicted respectively in Fig. 1. An electromagnetic field of a desired frequency and a desired field strength is generated by an electromagnetic wave source (3) which comprises one or more signal generators that are capable of generating electromagnetic waves, preferably sinusoidal waves, and preferably in the frequency range of 30 MHz - 3000 MHz. Such signal generators are well known in the art. Signal generators capable of generating signal with a narrower frequency range can also be used. If desirable, a signal amplifier can also be used to increase the output signal, and thus the strength of the EM field.

The electromagnetic field can be applied to the culture by a variety of means including placing the yeast cells in close proximity to a signal emitter connected to a source of electromagnetic waves. In one embodiment, the electromagnetic field is applied by signal emitters in the form of electrodes that are submerged in a culture of yeast cells (1). In a preferred embodiment, one of the electrodes is a metal plate, and the other electrode comprises a plurality of wires configured inside the container (2) so that the energy of the electromagnetic field can be evenly distributed in the culture. The number of electrode wires used depends on both the volume of the culture and the diameter of the wire. For example, for a culture having a volume of 5000 ml, one electrode wire having a diameter of between 0.1 to 1.2 mm can be used for each 100 ml of culture; for a culture having a volume greater than 1000 l, one electrode wire having a diameter of between 3 to 30 mm can be used for each 1000 l of culture. See Figure 1.

Without being bound by any theory or mechanism, the inventor believes that the culture conditions activate and/or enhance the expression of a gene or a set of genes in a yeast cell such that the cell becomes more efficient in performing certain metabolic activities which lead to the respective desired results.

In various embodiments, yeasts of the genera of *Saccharomyces, Schizosaccharomyces, Sporobolomyces, Torulopsis, Trichosporon, Wickerhamia, Ashbya, Blastomyces, Candida, Citeromyces, Crebrothecium, Cryptococcus, Debaryomyces, Endomycopsis; Geotrichum, Hansenula, Kloeckera, Lipomyces, Pichia, Rhodosporidium,* and *Rhodotorula* can be used in the invention.

Non-limiting examples of yeast strains include *Saccharomyces cerevisiae* Hansen, ACCC2034, ACCC2035, ACCC2036, ACCC2037, ACCC2038, ACCC2039, ACCC2040, ACCC2041, ACCC2042, AS2.1, AS2.4, AS2.11, AS2.14, AS2.16, AS2.56, AS2.69, AS2.70, AS2.93, AS2.98, AS2.101, AS2.109, AS2.110, AS2.112, AS2.139, AS2.173, AS2.174, AS2.182, AS2.196, AS2.242, AS2.336, AS2.346, AS2.369, AS2.374, AS2.375, AS2.379, AS2.380, AS2.382, AS2.390, AS2.393, AS2.395, AS2.396, AS2.397, AS2.398, AS2.399, AS2.400, AS2.406, AS2.408, AS2.409, AS2.413, AS2.414, AS2.415, AS2.416, AS2.422, AS2.423, AS2.430, AS2.431, AS2.432, AS2.451, AS2.452, AS2.453, AS2.458, AS2.460, AS2.463, AS2.467, AS2.486, AS2.501, AS2.502, AS2.503, AS2.504, AS2.516, AS2.535, AS2.536, AS2.558, AS2.560, AS2.561, AS2.562, AS2.576, AS2.593, AS2.594, AS2.614, AS2.620, AS2.628, AS2.631, AS2.666, AS2.982, AS2.1190, AS2.1364, AS2.1396, IFFI 1001, IFFI 1002, IFFI 1005, IFFI 1006, IFFI 1008, IFFI 1009, IFFI 1010, IFFI 1012, IFFI 1021, IFFI 1027, IFFI 1037, IFFI 1042, IFFI 1043, IFFI 1045, IFFI 1048, IFFI 1049, IFFI 1050, IFFI 1052, IFFI 1059, IFFI 1060, IFFI 1063, IFFI 1202, IFFI 1203, IFFI 1206, IFFI 1209, IFFI 1210, IFFI 1211, IFFI 1212, IFFI 1213, IFFI 1215, IFFI 1220, IFFI 1221, IFFI 1224, IFFI 1247, IFFI 1248, IFFI 1251, IFFI 1270, IFFI 1277, IFFI 1287, IFFI 1289, IFFI 1290, IFFI 1291, IFFI 1292, IFFI 1293, IFFI 1297, IFFI 1300, IFFI 1301, IFFI 1302, IFFI 1307, IFFI 1308, IFFI 1309, IFFI 1310, IFFI 1311, IFFI 1331, IFFI 1335, IFFI 1336, IFFI 1337, IFFI 1338, IFFI 1339, IFFI 1340, IFFI 1345, IFFI 1348, IFFI 1396, IFFI 1397, IFFI 1399, IFFI 1411, IFFI 1413; *Saccharomyces cerevisiae* Hansen Var. ellipsoideus (Hansen) Dekker, ACCC2043, AS2.2, AS2.3, AS2.8, AS2.53, AS2.163, AS2.168, AS2.483, AS2.541, AS2.559, AS2.606, AS2.607, AS2.611, AS2.612; *Saccharomyces chevalieri* Guillermond, AS2.131, AS2.213; *Saccharomyces delbrueckii*, AS2.285; *Saccharomyces delbrueckii* Lindner var. mongolicus Lodder et van Rij, AS2.209, AS2.1157; *Saccharomyces exiguus* Hansen, AS2.349, AS2.1158; *Saccharomyces fermentati* (Saito) Lodder et van Rij, AS2.286, AS2.343; *Saccharomyces logos* van laer et

Denamur ex Jorgensen, AS2.156, AS2.327, AS2.335; *Saccharomyces mellis* Lodder et Kreger Van Rij, AS2.195; *Saccharomyces microellipsoides* Osterwalder, AS2.699; Saccharomyces *oviformis* Osterwalder, AS2.100; *Saccharomyces rosei* (Guilliermond) Lodder et kreger van Rij, AS2.287; *Saccharomyces rouxii* Boutroux, AS2.178, AS2.180, AS2.370, AS2.371; *Saccharomyces sake* Yabe, ACCC2045; *Candida arborea*, AS2.566; *Candida Krusei* (Castellani) Berkhout, AS2.1045; *Candida lambica*(Lindner et Genoud) van.Uden et Buckley, AS2.1182; *Candida lipolytica* (Harrison) Diddens et Lodder, AS2.1207, AS2.1216, AS2.1220, AS2.1379, AS2.1398, AS2.1399, AS2.1400; *Candida parapsilosis* (Ashford) Langeron et Talice, AS2.590; *Candida parapsilosis* (Ashford) et Talice Var. intermedia Van Rij et Verona, AS2.491; *Candida pulcherriman* (Lindner) Windisch, AS2.492; *Candida rugousa* (Anderson) Diddens et Loddeer, AS2.511, AS2.1367, AS2.1369, AS2.1372, AS2.1373, AS2.1377, AS2.1378, AS2.1384; *Candida tropicalis* (Castellani) Berkout, ACCC2004, ACCC2005, ACCC2006, AS2.164, AS2.402, AS2.564, AS2.565, AS2.567, AS2.568, AS2.617, AS2.1387; *Candida utilis* Henneberg Lodder et Kreger Van Rij, AS2.120, AS2.281, AS2.1180; *Crebrothecium ashbyii* (Guillermond) Routein, AS2.481, AS2.482, AS2.1197; *Geotrichum candidum* Link, ACCC2016, AS2.361, AS2.498, AS2.616, AS2.1035, AS2.1062, AS2.1080, AS2.1132, AS2.1175, AS2.1183; *Hansenula anomala* (Hansen) H et P sydow, ACCC2018, AS2.294, AS2.295, AS2.296, AS2.297, AS2.298, AS2.299, AS2.300, AS2.302, AS2.338, AS2.339, AS2.340, AS2.341, AS2.470, AS2.592, AS2.641, AS2.642, AS2.635, AS2.782, AS2.794; *Hansenula arabitolgens* Fang, AS2.887; *Hansenula jadinii* Wickerham, ACCC2019; *Hansenula saturnus* (Klocker) H et P sydow, ACCC2020; *Hansenula schneggii* (Weber) Dekker, AS2.304; *Hansenula subpelliculosa* Bedford, AS2.738, AS2.740, AS2.760, AS2.761, AS2.770, AS2.783, AS2.790, AS2.798, AS2.866; *Kloeckera apiculata* (Reess emend. Klocker) Janke, ACCC2021, ACCC2022, ACCC2023, AS2.197, AS2.496, AS2.711, AS2.714; *Lipomyces starkeyi* Lodder et van Rij, ACCC2024, AS2.1390; *Pichia farinosa* (Lindner) Hansen, ACCC2025, ACCC2026, AS2.86, AS2.87, AS2.705, AS2.803; *Pichia membranaefaciens* Hansen, ACCC2027, AS2.89, AS2.661, AS2.1039; *Rhodosporidium toruloides* Banno, ACCC2028; *Rhodotorula glutinis* (Fresenius) Harrison, ACCC2029, AS2.280, ACCC2030, AS2.102, AS2.107, AS2.278, AS2.499, AS2.694, AS2.703, AS2.704, AS2.1146; *Rhodotorula minuta* (Saito) Harrison, AS2.277; *Rhodotorula rubar* (Demme) Lodder, ACCC2031, AS2.21, AS2.22, AS2.103, AS2.105, AS2.108, AS2.140, AS2.166, AS2.167, AS2.272, AS2.279, AS2.282; *Saccharomyces carlsbergensis* Hansen, ACCC2032, ACCC2033, AS2.113, AS2.116, AS2.118, AS2.121, AS2.132, AS2.162, AS2.189, AS2.200, AS2.216, AS2.265, AS2.377, AS2.417, AS2.420,

AS2.440, AS2.441, AS2.443, AS2.444, AS2.459, AS2.595, AS2.605, AS2.638, AS2.742, AS2.745, AS2.748, AS2.1042; *Saccharomyces uvarum Beijer*, IFFI 1023, IFFI 1032, IFFI 1036, IFFI 1044, IFFI 1072, IFFI 1205, IFFI 1207; *Saccharomyces willianus* Saccardo, AS2.5, AS2.7, AS2.119, AS2.152, AS2.293, AS2.381, AS2.392, AS2.434, AS2.614, AS2.1189; *Saccharomyces sp.*, AS2.311; *Saccharomyces ludwigii* Hansen, ACCC2044, AS2.243, AS2.508; *Saccharomyces sinenses* Yue, AS2.1395; *Schizosaccharomyces octosporus* Beijerinck, ACCC 2046, AS2.1148; *Schizosaccharomyces pombe* Linder, ACCC2047, ACCC2048, AS2.248, AS2.249, AS2.255, AS2.257, AS2.259, AS2.260, AS2.274, AS2.994, AS2.1043, AS2.1149, AS2.1178, IFFI 1056; *Sporobolomyces roseus* Kluyver et van Niel, ACCC 2049, ACCC 2050, AS2.619, AS2.962, AS2.1036, ACCC2051, AS2.261, AS2.262; *Torulopsis candida* (Saito) Lodder, ACCC2052, AS2.270; *Torulopsis famta* (Harrison) Lodder et van Rij, ACCC2053, AS2.685; *Torulopsis globosa* (Olson et Hammer) Lodder et van Rij, ACCC2054, AS2.202; *Torulopsis inconspicua* Lodder et van Rij, AS2.75; *Trichosporon behrendii* Lodder et Kreger van Rij, ACCC2055, AS2.1193; *Trichosporon capitatum* Diddens et Lodder, ACCC2056, AS2.1385; *Trichosporon cutaneum*(de Beurm et al.)Ota, ACCC2057, AS2.25, AS2.570, AS2.571, AS2.1374; *Wickerhamia fluoresens* (Soneda) Soneda, ACCC2058, AS2.1388.

Certain yeast species that can be activated or induced according to the present invention and are included in the present invention are known to be pathogenic to human and/or other living organisms, for example, *Ashbya gossypii*; *Blastomyces dermatitidis*; *Candida albicans*; *Candida parakrusei*; *Candida tropicalis*; *Citeromyces matritensis*; *Crebrothecium ashbyii*; *Cryptococcus laurentii*; *Cryptococcua neoformans*; *Debaryomyces hansenii*; *Debaryomyces kloeckeri*; *Debaryomyces* sp.; *Endomycopsis fibuligera*. Under certain circumstances, it may be less preferable to use such pathogenic yeasts in the biological compositions of the invention, for example, if such use is in an open field, it may endanger the health of human and/or other living organisms.

Yeasts of the *Saccharomyces* genus are generally preferred. Among strains of *Saccharomyces cerevisiae, Saccharomyces cerevisiae* Hansen is a preferred strain. The most preferred strains of yeast are *Saccharomyces cerevisiae* strains having accession numbers AS2.504, AS2.558, AS2.413, AS2.397, AS2.69, AS2.109, AS2.607, AS2.516, AS2.561, AS2.422, AS2.393, AS2.631, AS2.982, AS2.560, AS2.467, AS2.415, AS2.375, AS2.628, AS2.1190, AS2.562, AS2.463, AS2.409, AS2.379, AS2.666, AS2.631, AS2.182, AS2.431, AS2.606, AS2.53, AS2.611, AS2.414, AS2.576, AS2.483, IFFI 1211, 1FF1 1293, IFFI 1308, IFFI 1210, IFFI 1213, IFFI 1307, IFFI 1206, IFFI 1052, IFFI 1301, IFFI 1291, IFFI 1202, IFFI 1021, IFFI 1059, IFFI 1052, IFFI 1441, IFFI 1008, IFFI 1220, IFFI 1302,

and IFFI 1023 as deposited at the China General Microbiological Culture Collection Center (CGMCC).

Generally, yeast strains useful for the invention can be obtained from private or public laboratory cultures, or publically accessible culture deposits, such as the American Type Culture Collection, 10801 University Boulevard, Manassas, VA 20110-2209 and the China General Microbiological Culture Collection Center (CGMCC), China Committee for Culture Collection of Microorganisms, Institute of Microbiology, Chinese Academy of Sciences, Haidian, P.O. Box 2714, Beijing, 100080, China.

Although it is preferred, the preparation of the yeast cell components of the invention is not limited to starting with a pure strain of yeast. Each yeast cell component may be produced by culturing a mixture of yeast cells of different species or strains. The constituents of a yeast cell component can be determined by standard yeast identification techniques well known in the art.

The ability and efficiency of the activated yeasts to perform a desired function before or after culturing under the conditions of the invention can readily be tested by methods known in the art. For example, HPLC or mass spectrometry can be used for detecting and analyzing various organic molecules in a sample of solid waste. Microbiological methods well known in the art can be used for detecting and counting the number of viable microorganisms and the total number of microorganisms in a sample.

When treating organic manure that has a relatively high bacteria count, the biological composition can be formulated to comprise largely yeast cells that suppress bacterial growth. When the biological composition is to be used to treat solid waste that is contaminated with undesirable chemicals, the biological composition can be formulated to comprise mostly yeast cells that degrade undesirable chemicals. Thus, the biological compositions have utility in many types of situations encountered in municipal, commercial, agricultural, and industrial establishments. The invention can also be used domestically, especially in rural areas.

The biological compositions of the invention can be applied directly to the solid waste. As known to those skilled in the relevant art, many methods and appliances may be used to mix the yeasts with the solid waste. In one embodiment, a culture broth of the yeasts of the present invention are added directly to the solid waste to be treated. In another embodiment, dried powders of the yeasts of the present invention are mixed with the solid waste to which water is added at a later time. The biological compositions may be applied to and mixed with the solid waste by spreaders, sprayers, and other mechanized means which may be automated. The amount of biological composition used depends in

part on the circumstances and the type of solid waste, and can be determined empirically. However, to achieve an effective treatment, it is desired to use from about 300 to 600g dry weight (less than 10% moisture) of the biological composition per cubic meter of solid waste. The yeast cells are first mixed with water in the ratio of about 1000 g yeasts (dry weight) to about 30 liters, and then incubated for 12 to 24 hours, prior to application to the solid waste. The benefit of the treatment, e.g., reduction of odor or bacteria count, takes effect about 24 to 72 hours after the application. Although not necessary, the biological compositions of the invention can also be used in conjunction or in rotation with other types of deodorants, disinfectants, and detoxification agents.

Described respectively in Sections 5.1 - 5.4 are the yeast cell components used for degradation of antibiotics, pathogen suppression, degradation of undesirable chemicals, and reduction of odor. Methods for preparing each yeast cell components are described. Section 5.6 describes the manufacture of the biological compositions of the invention. In various embodiments of the invention, standard techniques for handling, transferring, and storing yeasts are used. Although it is not necessary, sterile conditions or clean environments are desirable when carrying out the manufacturing processes of the invention.

5.1. YEAST CELLS THAT DEGRADE ANTIBIOTICS

The present invention provides yeast cells that are capable of degrading antibiotics that are typically found in manures and sludge.

According to the invention, the ability of yeast cells to degrade antibiotics is activated or enhanced by culturing the yeast cells in the presence of an electromagnetic field in an appropriate culture medium. The resulting yeast cells can be used as a component in the biological solid waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to degrade antibiotics can generally be found in the range of 70 MHz to 600 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their enhanced ability to decompose one or more types of antibiotics by methods well known in the art. Antibiotics degraded by the yeasts of the invention include but are not limited to molecules within the families of beta-lactams, tetracyclines, polypeptides, glycopeptides, aminoglycosides, and macrolides.

The method of the invention for making antibiotics-degrading yeasts is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose,

fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(NH_4)_2HPO_4$, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

Table 1: Composition for a culture medium for yeasts that degrade antibiotics

Medium Composition	Quantity
Manure or sludge	8.0g, dry weight, >120 mesh
NaCl	0.2g
$MgSO_4 \cdot 7H_2O$	0.2g
$CaCO_3 \cdot 5H_2O$	0.5g
$CaSO_4 \cdot 2H_2O$	0.2g
Peptone	1.5g
K_2HPO_4	0.5g
Extract containing antibiotics (≥ 100 ug/ml)	600ml
Autoclaved water	400ml

The extract containing antibiotics is prepared by dispersing and incubating 500g of fresh waste, e.g., manures, sludge, in about 600ml of warm water (at 35-40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. If the extract contains only negligible amount of a particular antibiotics, an appropriate amount of the antibiotics can be added into the extract.

It should be noted that the composition of the media provided in Table 1 is not intended to be limiting. Various modifications of the culture medium may be made by

those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2-10^5 cell/ml, preferably $3x10^2$-10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 70.000 to 100.000 MHz and 410.000 to 620.000 MHz, preferably. The field strength of the EM field(s) is in the range of 40 to 250 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 144 - 384 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of 8.5-85mV/cm, usually at about 50 mV/cm, is used. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 150-250 mV, usually to about 200 mV. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m^3, preferably 0.4 mol/m^3. The oxygen level can be controlled by any

conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C. The recovered yeast cells may also be dried and stored in powder form.

To determine the activity of the activated yeast cells towards an antibiotic compound, methods well known in the art, such as HPLC, can be used to measure the amounts of the antibiotic compound in a test sample at various time point and under different incubation conditions. For example, a sample containing known concentration of an antibiotic (up to 100 mg per liter) is prepared. Then, 0.1 ml of activated and unactivated yeasts (at least 10^7 cells/ml) are added to the 100 liter samples containing the antibiotics, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the extracts are determined and compared by performing HPLC on the samples.

The method is generally applicable to many type of antibiotics. In specific embodiments, methods that are optimized for a particular type of antibiotics are described, infra.

Yeast cell component that decomposes penicillins

In a specific embodiment, a method for producing yeast cells that decompose penicillins, e.g., penicillin G and Cloxacillin, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 100.000 MHz, including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, and 100 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.399 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 77 MHz at 48 mV/cm for 15 h; 83 MHz at 48 mV/cm for 15 h; 90 MHz at 48 mV/cm for 15 h; 96 MHz at 48 mV/cm for 15 h; 77 MHz at 200 mV/cm for 30 h; 83 MHz at 200 mV/cm for 30 h; 90 MHz at 200 mV/cm for 30 h; 96 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards penicillin was determined by measuring the amounts of penicillin the activated yeast cells can degrade. Two 100 liter samples each containing a penicillin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of

antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of penicillin in the sample with the activated yeast cells was reduced by more than 56.5%.

Yeast cell component that decomposes chlortetracycline

In a specific embodiment, a method for producing yeast cells that decompose chlortetracycline, e.g., aureomycin, chlortetracycline hydrochloridum, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.748 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards chlorotetracycline was determined by measuring the amounts of chlorotetracycline the activated yeast cells can degrade. Two 100 liter samples each containing a chlorotetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the two samples. Comparing to samples with unactivated yeast cells, the amount of chlorotetracycline in the samples with the activated yeast cells was reduced by more than 62.3%.

Yeast cell component that decomposes oxytetracycline

In a specific embodiment, a method for producing yeast cells that decompose oxytetracycline, e.g., oxytetracycline hydrochloridum, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, Yeast cells of *Saccharomyces cerevisiae* strain AS2.101 are cultured at about 25-30°C in a culture

medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 74 MHz at 48 mV/cm for 15 h; 88 MHz at 44 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 74 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards oxytetracycline was determined by measuring the amounts of oxytetracycline the activated yeast cells can degrade. Two 100 liter samples each containing an oxytetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with Comparing to samples unactivated yeast cells, the amount of oxytetracycline in the samples with the activated yeast cells was reduced by more than 65.5%.

Yeast cell component that decomposes doxycycline

In a specific embodiment, a method for producing yeast cells that decompose doxycycline is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.417 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 77 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 71 MHz at 200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 77 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards doxycycline was determined by measuring the amounts of doxycycline the activated yeast cells can degrade. Two 100 liter samples each containing a doxycycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples are determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the

amount of doxycycline in the samples with the activated yeast cells was reduced by more than 54.9%.

Yeast cell component that decomposes tetracycline

In a specific embodiment, a method for producing yeast cells that decompose tetracycline is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.70 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 75 MHz at 48 mV/cm for 15 h; 82 MHz at 48 mV/cm for 15 h; 85 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 75 MHz at 200 mV/cm for 30 h; 82 MHz at 200 mV/cm for 30 h; 85 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards tetracycline was determined by measuring the amounts of tetracycline the activated yeast cells can degrade. Two 100 liter samples each containing a tetracycline concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples are determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of tetracycline in the samples with the activated yeast cells was reduced by more than 67.6%.

Yeast cell component that decomposes streptomycin

In a specific embodiment, a method for producing yeast cells that decompose streptomycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.441 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 73 MHz at 48 mV/cm for 15 h; 80 MHz at 48 mV/cm for 15 h; 96 MHz at 48 mV/cm for 15 h; 70 MHz at

200 mV/cm for 30 h; 73 MHz at 200 mV/cm for 30 h; 80 MHz at 200 mV/cm for 30 h; 96 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards streptomycin was determined by measuring the amounts of streptomycin the activated yeast cells can degrade. Two 100 liter samples each containing a streptomycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of streptomycin in the samples with the activated yeast cells was reduced by more than 77.8%.

Yeast cell component that decomposes kanamycin

In a specific embodiment, a method for producing yeast cells that decompose kanamycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, east cells of *Saccharomyces cerevisiae* strain AS2.336 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 71 MHz at 48 mV/cm for 15 h; 78 MHz at 48 mV/cm for 15 h; 86 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 71 MHz at 200 mV/cm for 30 h; 78 MHz at 200 mV/cm for 30 h; 86 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards kanamycin was determined by measuring the amounts of kanamycin the activated yeast cells can degrade. Two 100 liter samples each containing a kanamycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of kanamycin in the samples with the activated yeast cells was reduced by more than 68.7%.

Yeast cell component that decomposes erythromycin

In a specific embodiment, a method for producing yeast cells that decompose erythromycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.422 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 73 MHz at 48 mV/cm for 15 h; 79 MHz at 48 mV/cm for 15 h; 88 MHz at 48 mV/cm for 15 h; 98 MHz at 48 mV/cm for 15 h; 73 MHz at 200 mV/cm for 30 h; 79 MHz at 200 mV/cm for 30 h; 88 MHz at 200 mV/cm for 30 h; 98 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards erythromycin was determined by measuring the amounts of erythromycin the activated yeast cells can degrade. Two 100 liter samples each containing a erythromycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of erythromycin in the samples with the activated yeast cells was reduced by more than 72.7%.

Yeast cell component that decomposes spiramycin

In a specific embodiment, a method for producing yeast cells that decompose spiramycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.620 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 70 MHz at 48 mV/cm for 15 h; 77 MHz at 48 mV/cm for 15 h; 84 MHz at 48 mV/cm for 15 h; 93 MHz at 48 mV/cm for 15 h; 70 MHz at 200 mV/cm for 30 h; 77 MHz at 200 mV/cm for 30 h; 84 MHz at 200 mV/cm for 30 h; 93 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards spiramycin was determined by measuring the amounts of spiramycin the activated yeast cells can degrade. Two 100 liter samples each containing a spiramycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) are added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of spiramycin in the samples with the activated yeast cells was reduced by more than 66.8%.

Yeast cell component that decomposes bacitracin

In a specific embodiment, a method for producing yeast cells that decompose bacitracin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz, including but not limited to 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.486 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 75 MHz at 48 mV/cm for 15 h; 78 MHz at 48 mV/cm for 15 h; 81 MHz at 48 mV/cm for 15 h; 95 MHz at 48 mV/cm for 15 h; 75 MHz at 200 mV/cm for 30 h; 78 MHz at 200 mV/cm for 30 h; 81 MHz at 200 mV/cm for 30 h; 95 MHz at 200 mV/cm for 30 h.

The activity of the activated yeast cells towards bacitracin was determined by measuring the amounts of bacitracin the activated yeast cells can degrade. Two 100 liter samples each containing a bacitracin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of bacitracin in the samples with the activated yeast cells was reduced by more than 71.6%.

Yeast cell component that decomposes colistin

In a specific embodiment, a method for producing yeast cells that decompose colistin or colistin sulfate is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.611 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 433 MHz at 85 mV/cm for 12 h; 440 MHz at 85 mV/cm for 12 h; 446 MHz at 85 mV/cm for 12 h; 457 MHz at 85 mV/cm for 12 h; 433 MHz at 204 mV/cm for 24 h; 440 MHz at 204 mV/cm for 24 h; 446 MHz at 204 mV/cm for 24 h; 457 MHz at 204 mV/cm for 24 h.

The activity of the activated yeast cells towards colistin was determined by measuring the amounts of colistin the activated yeast cells can degrade. Two 100 liter samples each containing a colistin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which does not contain any yeast cells: After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of colistin in the samples with the activated yeast cells was reduced by more than 71.6%.

Yeast cell component that decomposes chloramphenicol

In a specific embodiment, a method for producing yeast cells that decompose chloramphenicol and salts such as chloromycetin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.371 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 419 MHz at 85 mV/cm for 12 hr; 425 MHz at 85 mV/cm for 12 h; 433 MHz at 85 mV/cm for 12 h; 462 MHz at 85 mV/cm for 12 h; 419 MHz at183 mV/cm for 24 h; 425 MHz at183 mV/cm for 24 h; 433 MHz at183 mV/cm for 24 h; 462 MHz at183 mV/cm for 24 h.

The activity of the activated yeast cells towards chloramphenicol was determined by measuring the amounts of chloramphenicol the activated yeast cells can degrade. Two 100 liter samples each containing a chloramphenicol concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of chloramphenicol in the samples with the activated yeast cells was reduced by more than 58.6%.

Yeast cell component that decomposes cephalosporins

In a specific embodiment, a method for producing yeast cells that decompose cephalosporins, e.g., cephalothin, cephaloridine, cephaloglyin, cephalolexin, and cephazoline, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 410.000 to 470.000 MHz, including but not limited to 430, 431, 432, 433, 434, 435, 436, 437, 438, 439, 440, 441, 442, 443, 444, 445, 446, 447, 448, 449, 450, 451, 452, 453, 454, 456, 457, 458, 459, and 460 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.559 were cultured at about 25-30 °C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 434 MHz at 85 mV/cm for 12 h, 441 MHz at 85 mV/cm for 12 h, 450 MHz at 85 mV/cm for 12 h, 458 MHz at 85 mV/cm for 12 h; 434 MHz at 198 mV/cm for 24 h, 441 MHz at 198 mV/cm for 24 h, 450 MHz at 198 mV/cm for 24 h, 458 MHz at 198 mV/cm for 24 h.

The activity of the activated yeast cells towards cephalosporins was determined by measuring the amounts of cephalothin the activated yeast cells can degrade. Two 100 liter samples each containing a cephalothin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of cephalothins in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes neomycin

In a specific embodiment, a method for producing yeast cells that decompose neomycin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 550.000 to 620.000 MHz, including but not limited to 555, 556, 557, 558, 559, 560, 561, 562, 563, 564, 565, 566, 567, 568, 569, 570, 571, 572, 573, 574, and 575 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.182 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 557 MHz at 85 mV/cm for 12 h, 564 MHz at 85 mV/cm for 12 h, 568 MHz at 85 mV/cm for 12 h, 574 MHz at 85 mV/cm for 12 h; 557 MHz at 231 mV/cm for 24 h, 564 MHz at 231 mV/cm for 24 h, 568 MHz at 231 mV/cm for 24 h, 574 MHz at 231 mV/cm for 24 h.

The activity of the activated yeast cells towards neomycin was determined by measuring the amounts of neomycin the activated yeast cells can degrade. Two 100 liter samples each containing a neomycin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of neomycin in the samples with the activated yeast cells was reduced by more than 67.7%.

Yeast cell component that decomposes novobiocin

In a specific embodiment, a method for producing yeast cells that decompose novobiocin is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 550.000 to 620.000 MHz, including but not limited to 590, 591, 592, 593, 594, 595, 596, 597, 598, 599, 600, 601, 602, 603, 604, 605, 606, 607, 608, 609, and 610 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.112 were cultured at about 25-30°C in a culture medium as described in Table 1 in the presence of a series of 8 EM fields in the order stated: 594 MHz at 85 mV/cm for 12 h, 599 MHz at 85 mV/cm for 12 h, 602 MHz at 85 mV/cm for 12 h, 608 MHz at 85 mV/cm for 12 h; 594 MHz at 231 mV/cm for 24 h, 599 MHz at 231 mV/cm for 24 h, 602 MHz at 231 mV/cm for 24 h, 608 MHz at 231 mV/cm for 24 h.

The activity of the activated yeast cells towards novobiocin was determined by measuring the amounts of novobiocin the activated yeast cells can degrade. Two 100 liter samples each containing a novobiocin concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of novobiocin in the samples with the activated yeast cells was reduced by more than 69.5%.

5.2. YEAST CELL COMPONENTS THAT DECOMPOSE UNDESIRABLE CHEMICALS

The present invention further provides yeast cells that are capable of degrading chemicals that are typically found in solid waste.

According to the invention, the ability of yeast cells to degrade undesirable chemicals is activated or enhanced by culturing the yeast cells in the presence of an electromagnetic field in an appropriate culture medium. The resulting yeast cells can be used as a component in the biological waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to degrade undesirable chemicals can generally be found in the range of 30 to 280 MHz, 410 to 440 MHz, 660 to 690 MHz, 1400 to 1435 MHz, and 1980 to 2210 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their enhanced ability to decompose one or more types of chemicals by methods well known in the art. Undesirable chemicals degraded by the yeasts of the invention include but are not limited to herbicides, pesticides, and fertilizer-related pollutants.

The method of the invention for making chemical-degrading yeasts is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5%

and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(NH_4)_2HPO_4$, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

Table 2: Composition for a culture medium for yeasts that degrade chemicals

Medium Composition	Quantity
Manure or sludge	8.0g, dry weight, >120 mesh
NaCl	0.2g
$MgSO_4 \cdot 7H_2O$	0.2g
$CaCO_3 \cdot 5H_2O$	0.5g
$CaSO_4 \cdot 2H_2O$	0.2g
Peptone	1.5g
K_2HPO_4	0.5g
Extract containing chemicals (≥ 100 ug/ml)	600ml
Autoclaved water	400ml

The extract for the culture medium is prepared by incubating 500g of fresh waste, e.g., manures, sludge, and/or garbage, in about 600ml of warm water (at 35-40°C) for 24 hours at 30-37°C, and filtering the fluid to remove particulate matters. If the extract contains low amount of the particular chemicals, an appropriate amount of the chemical can be added into the extract.

It should be noted that the composition of the media provided in Table 2 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2-10^5 cell/ml, preferably $3x10^2$-10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series

of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the ranges of 30.000 to 100.000, 70.000 to 280.000, 410.000 to 430.000, 660.000 to 680.000 and 1980.000 to 2210.000 MHz. The field strength of the EM field(s) is in the range of 40 to 250 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 90 - 480 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of about 8 to about 300 mV/cm is used. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m^3, preferably 0.4 mol/m^3. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0°C to 4°C. The recovered yeast cells may also be dried and stored in powder.

To determine the activity of the activated yeast cells towards a chemical compound, methods well known in the art, such as HPLC, can be used to measure the amounts of the compound in a test sample at various time point and under different incubation conditions. For example, a sample containing a known concentration of a chemical compound (up to 100 mg per liter) is prepared. Then, 0.1 ml of activated and

unactivated yeasts (at least 10^7 cells/ml) were added to the 100 liter samples containing the compound, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of compound remaining in the extracts are determined and compared by performing HPLC on the samples.

The method is generally applicable to many classes of chemicals. In specific embodiments, method that are optimized for a particular class of chemicals are described, infra.

Yeast cell component that decomposes aromatic compounds

In specific embodiment, method for producing yeast cells that decompose trichlorophenol, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 30.000 to 100.000 MHz, or preferably 52 to 98 MHz including but not limited to 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98 MHz. Yeast cells of *Saccharomyces cerevisiae* strain IFFI1411 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 6 EM fields in the order stated: 82 MHz at 82 mv/cm for 25 h; 90 MHz at 82 mv/cm for 25 h; 98 MHz at 82mv/cm for 25 h; 82 MHz at 274 mv/cm for 32 h; 90 MHz at 274 mv/cm for 32 h; 98 MHz at 274 mv/cm for 25 h.

The activity of the activated yeast cells towards trichlorophenol was determined by measuring the amounts of the compound the activated yeast cells can degrade. Two 100 liter samples each containing the compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of the compound remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of tripchlorophenol in the samples with the activated yeast cells was reduced by more than 56.4%.

In another specific embodiment, method for producing yeast cells that decompose toluene or ethylbenzene, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 52.000 to 98.000 MHz, including but not limited to 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.56 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a

series of 4 EM fields in the order stated: 76 MHz at 89 mV/cm for 20 h; 80 MHz at 89 mV/cm for 200 h; 86 MHz at 89 mV/cm for 20 h; and 96 MHz at 89 mV/cm for 20 h.

The activity of the activated yeast cells towards toluene or ethylbenzene was determined by measuring the amounts of the compounds the activated yeast cells can degrade. Two 100 liter samples each containing the compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of the compound remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of toluene in the samples with the activated yeast cells was reduced by more than 74.3%.

In another specific embodiment of the invention, a method for producing yeast cells that decompose dimethylbenzene compounds, e.g., p-xylene, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 30.000 to 50.000 MHz, or 70.000 to 98.000 MHz including but not limited to 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.420 are cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 72 MHz at 93 mV/cm for 20 h; 80 MHz at 93 mV/cm for 20 h; 88 MHz at 93 mV/cm for 20 h; and 98 MHz at 93 mV/cm for 20 h.

The activity of the activated yeast cells towards dimethylbezene compounds was determined by measuring the amounts of dimethylbezene compounds the activated yeast cells can degrade. Two 100 liter samples each containing a dimethylbenzene compound at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of p-xylene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of p-xylene in the samples with the activated yeast cells was reduced by more than 66.6%.

Yeast cell component that decomposes aldehyde compounds

In another specific embodiment of the invention, a method for producing yeast cells that decompose benzaldehyde and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 133-151 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, or 151 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.374 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 130 mV/cm for 30 h; 86 MHz at 130 mV/cm for 30 h; 94 MHz at 130 mV/cm for 30 h; 96 MHz at 130 mV/cm for 30 h.

The activity of the activated yeast cells towards benzaldehyde was determined by measuring the amounts of benzaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing benzaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of benzaldehyde remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of benzaldehyde in the samples with the activated yeast cells was reduced by more than 63.6%.

In yet another specific embodiment, a method for producing yeast cells that decompose propylaldehyde and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 145-162 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 146, 147, 148, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, and 162 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.414 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 103 mV/cm for 20 h; 88 MHz at 103 mV/cm for 20 h; 96 MHz at 103 mV/cm for 20 h; 98 MHz at 103 mV/cm for 30 h.

The activity of the activated yeast cells towards propylaldehyde was determined by measuring the amounts of propylaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing propylaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast

cells. After 24 hours, the amounts of propylaldehyde remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of propylaldehyde in the samples with the activated yeast cells was reduced by more than 73.8%.

In yet another specific embodiment, a method for producing yeast cells that decompose nenanthaldehyde compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 MHz or 100.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, and 98 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.503 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 81 MHz at 90 mV/cm for 12 h, 85 MHz at 90 mV/cm for 12 h, 89 MHz at 90 mV/cm for 12 h, 94 MHz at 90 mV/cm for 12 h, 81 MHz at 157 mV/cm for 24 h, 85 MHz at 157 mV/cm for 24 h, 89 MHz at 157 mV/cm for 24 h, 94 MHz at 157 mV/cm for 24 h.

The activity of the activated yeast cells towards nenanthaldehyde was determined by measuring the amounts of nenanthaldehyde the activated yeast cells can degrade. Two 100 liter samples each containing nenanthaldehyde at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of nenanthaldehyde remaining in the samples were determined and compared by performing HPLC on the two samples. Comparing to samples with unactivated yeast cells, the amount of nenanthaldehyde in the samples with the activated yeast cells was reduced by more than 81.3% in 24 hours.

<u>Yeast cell component that decomposes halogen-substituted benzene compounds</u>

In a specific embodiment, method for producing yeast cells that decompose halogen-substituted benzene compounds, e.g., m-dichlorobenzene, is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 163.000 to 183.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 163, 164, 165, 166, 167, 168, 169, 170, 171, 172, 173, 174, 175, 176, 177, 178. 179, 180, 181, 182, and 183 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.483 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order

stated: 72 MHz at 107 mV/cm for 20 h; 80 MHz at 107 mV/cm for 10 h; 90 MHz at 107 mV/cm for 30 h; 94 MHz at 107 mV/cm for 40 h.

The activity of the activated yeast cells towards dichlorobenzene was determined by measuring the amounts of dichlorobenzene the activated yeast cells can degrade. Two 100 liter samples each containing dichlorobenzene concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dichlorobenzene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dichlorobenzene in the samples with the activated yeast cells was reduced by more than 64.6%.

Yeast cell component that decomposes acetophenone and related compounds.

In yet another embodiment, a method for producing yeast cells that decompose acetophenone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 175.000 to 191.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 175. 176, 177, 178, 179, 180, 181, 182, 183, 184, 185, 186, 187, 188, 189, 190, and 191 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.265 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 124 mV/cm for 20 h; 82 MHz at 124 mV/cm for 30 h; 90 MHz at 124 mV/cm for 40 h; 98 MHz at 124 mV/cm for 20 h.

The activity of the activated yeast cells towards acetophenone was determined by measuring the amounts of acetophenone the activated yeast cells can degrade. Two 100 liter samples each containing acetophenone at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of acetophenone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of acetophenone compounds in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes arsanilic acid and related compounds

In yet another embodiment, a method for producing yeast cells that decompose arsanilic acid and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 183.000 to 205.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 183, 184, 185, 186, 187, 188, 189, 190, 191, 192, 193, 194, 195, 196, 197, 198, 199, 200, 201, 202, 203, 204, and 205 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.745 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 133 mV/cm for 30 h; 88 MHz at 133 mV/cm for 40 h; 92 MHz at 133 mV/cm for 30 h; 96MHz at 133 mV/cm for 30 h.

The activity of the activated yeast cells towards arsanilic acid was determined by measuring the amounts of arsanilic acid compounds the activated yeast cells can degrade. Two 100 liter samples each containing an arsanilic acid at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of arsanilic acid antibiotics remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of arsanilic acid in the samples with the activated yeast cells was reduced by more than 75.5%.

Yeast cell component that decomposes roxarsone and related compounds

In another specific embodiment, a method for producing yeast cells that decompose roxarsone and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 114.000 to 128.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125, 126, 127, and 128 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.173 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 110 mV/cm for 10 h; 92 MHz at 110 mV/cm for 10h; 78 MHz at 213 mV/cm for 30 h; 92 MHz at 213 mV/cm for 30 h.

The activity of the activated yeast cells towards roxarsone was determined by measuring the amounts of roxarsone the activated yeast cells can degrade. Two 100 liter samples each containing roxarsone concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of roxarsone remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of roxarsone in the samples with the activated yeast cells was reduced by more than 67.9%.

Yeast cell component that decomposes furazolidonum compounds

In yet another specific embodiment, a method for producing yeast cells that decompose furazolidonum and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 200.000 to 220.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 200, 201, 202, 203, 204, 205, 206, 207, 208, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, and 220 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.397 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 74 MHz at 98 mV/cm for 30 h; 76 MHz at 98 mV/cm for 20 h; 86 MHz at 98 mV/cm for 30 h; 94 MHz at 98 mV/cm for 30 h.

The activity of the activated yeast cells towards furazolidonum was determined by measuring the amounts of furazolidonum the activated yeast cells can degrade. Two 100 liter samples each containing furazolidonum concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of furazolidonum remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of furazolidonum in the samples with the activated yeast cells was reduced by more than 81.4%.

Yeast cell component that decomposes Decoquinate

In yet another specific embodiment, a method for producing yeast cells that decompose decoquinate and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 213.000 to 229.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 210, 211, 212, 213, 214, 215, 216, 217, 218, 219, 220, 221, 222, 223, 224, 225, 226, 227, 228, and 229 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.452 were cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 78 MHz at 112 mV/cm for 30 h; 82 MHz at 112 mV/cm for 30 h; 86 MHz at 112 mV/cm for 30 h; 94 MHz at 112 mV/cm for 20 h.

The activity of the activated yeast cells towards decoquinate was determined by measuring the amounts of decoquinate the activated yeast cells can degrade. Two 100 liter samples each containing decoquinate concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of decoquinate remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of decoquinate in the samples with the activated yeast cells was reduced by more than 67.9%.

Yeast cell component that decomposes Trichlorophonum compounds

In yet another specific embodiment, a method for producing yeast cells that decompose trichlorophonum and related compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 220.000 to 250.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, and 250 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.100 were cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 74 MHz at 219 mV/cm for 30 h; 86 MHz at 219 mV/cm for 20 h; 96 MHz at 219 mV/cm for 30 h; 98 MHz at 219 mV/cm for 20 h.

The activity of the activated yeast cells towards trichlorophonum was determined by measuring the amounts of trichlorophonum the activated yeast cells can degrade. Two 100 liter samples each containing trichlorophonum concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated

yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of trichlorophonum remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of trichlorophonum in the samples with the activated yeast cells was reduced by more than 72.4%.

Yeast cell component that decomposes Dinitolmide

In a specific embodiment, method for producing yeast cells that decompose dinitolmide and related compounds is provided. Dinitolmide is 2-methyl-3,5-dinitrobenzamide and is also known as zoalene. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 70.000 to 98.000 MHz or 220.000 to 250.000 MHz including but not limited to 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 97, 98, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, 246, 248, and 250 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.189 are cultured at about 25-30 °C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 76 MHz at 202 mV/cm for 30 h; 82 MHz at 202 mV/cm for 30 h; 90 MHz at 202 mV/cm for 20 h; 96 MHz at 202 mV/cm for 20 h.

The activity of the activated yeast cells towards dinitolmide was determined by measuring the amounts of zoalene compounds the activated yeast cells can degrade. Two 100 liter samples each containing a dinitolmide concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dinitolmide remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dinitolmide in the samples with the activated yeast cells was reduced by more than 72.4%.

Yeast cell component that removes ammonium compound (NH_4)

In a specific embodiment, a method for producing yeast cells that remove or reduce the level of ammonium compounds in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 660 to 680 MHz or 2160

to 2190 MHz, and preferably 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, 680, 2170, 2171, 2172, 2173, 2174, 2175, 2176, 2177, 2178, 2179, 2180, 2181, 2182, 2183, 2184, 2185 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.614 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 662 MHz at 152 mv/cm for 18 h; 666 MHz at 152 mv/cm for 18 h; 672 MHz at 152 mv/cm for 18 h; 678 MHz at 152 mv/cm for 18 h; 662 MHz at 310 mv/cm for 25 h; 666 MHz at 310 mv/cm for 25 h; 672 MHz at 310 mv/cm for 35 h; 678 MHz at 310 mv/cm for 35 h.

The activity of the activated yeast cells was determined by measuring the amounts of ammonium compounds removed by the activated yeast cells. The amount of ammonium compounds in the samples with the activated yeast cells was reduced significantly (>93.6%) compared to the sample containing unactivated yeast cells.

Yeast cell component that removes nitrates and nitrites

In a specific embodiment, a method for producing yeast cells that remove or reduce the level of nitrates and nitrites in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 661.000 to 680.000 MHz including but not limited to 661, 662, 663, 664, 665, 666, 667, 668, 669, 670, 671, 672, 673, 674, 675, 676, 677, 678, 679, and 680 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.14 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 661 MHz at 126 mV/cm for 25 h; 665 MHz at 126 mV/cm for 25 h; 672 MHz at 126 mV/cm for 25 h; 676 MHz at 126 mV/cm for 25 h; 661 MHz at 196 mV/cm for 25 h; 665 MHz at 196 mV/cm for 25 h; 672 MHz at 196 mV/cm for 38 h; 676 MHz at 196 mV/cm for 38 h.

The activity of the activated yeast cells towards nitrates was determined by measuring the amounts of nitrates removed by the activated yeast cells. Two 100 liter samples each containing nitrates at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of nitrates remaining in the samples were determined and compared by performing HPLC

on the samples. Comparing to samples with unactivated yeast cells, the amount of nitrates in the samples with the activated yeast cells was reduced by more than 69.7%

Yeast cell component that remove biologically available phosphorus

In a specific embodiment, a method for producing yeast cells that remove biologically available phosphorus, e.g., HPO_4^{2-}, $H_2PO_4^-$, etc., is provided. In a specific embodiment, a method for producing yeast cells that remove or reduce the level of nitrates and nitrites in solid waste is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 80.000 to 440.000 MHz, preferably 86.000 to 120.000 MHz or 410.000 to 440.000 MHz including but not limited to 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 410, 411, 412, 413, 414, 415, 416, 417, 418, 419, 420, 421, 422, 423, 424, 425, 426, 427, 428, 429, and 430 MHz. For example, Yeast cells of *Saccharomyces cerevisiae* strain AS2.620 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 98 MHz at 68 mv/cm for 24 h; 112 MHz at 68 mv/cm for 24 h; 108 MHz at 68 mv/cm for 24 h; 118 MHz at 68 mv/cm for 24 h; 98 MHz at 240 mv/cm for 24 h; 112 MHz at 240 mv/cm for 24 h; 108 MHz at 240 mv/cm for 42 h; 118 MHz at 240 mv/cm for 42 h.

The activity of the activated yeast cells towards available phosphorus was determined by measuring the amounts of available phosphorus the activated yeast cells can remove. Two 100 liter samples each containing available phosphorus concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of phosphorous remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of available phosphorus in the samples with the activated yeast cells was reduced by more than 65.8%.

Yeast cell component that decomposes Trichlorphon

In a specific embodiment, a method for producing yeast cells that decompose trichlorphon and related organophosphate pesticide compounds is provided. The

frequencies of the EM field(s) used to activate the yeast cells are in the range of 1980.000 to 2020.000, and preferably 2000.000 to 2020.000 including but not limited to 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019, and 2020 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.440 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 2000 MHz at 125mv/cm for 10 h; 2004 MHz at 125 mv/cm for 10 h; 2009 MHz at 125 mv/cm for 24 h; 2018 MHz at 125 mv/cm for 24 h; 2000 MHz at 168 mv/cm for 10 h; 2004 MHz at 168mv/cm for 10 h; 2009 MHz at 168 mv/cm for 56 h; 2018 MHz at 168 mv/cm for 56 h.

The activity of the activated yeast cells towards trichlorphon was determined by measuring the amounts of trichlorphon the activated yeast cells can degrade. Two 100 liter samples each containing trichlorphon concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of trichlorphon remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of trichlorphon in the samples with the activated yeast cells was reduced by more than 10% in 48 hours.

Yeast cell component that decomposes Dichlorvos

In a specific embodiment, a method for producing yeast cells that decompose dichlorvos (DDVP) and related organophosphate pesticide compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.443 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1993 MHz at 140 mV/cm for 24 h; 2023 MHz at 140 mV/cm for 24 h; 2083 MHz at 140 mV/cm for 24 h; 2103 MHz at 140 mV/cm for 24 h; 1993 MHz at 190 mV/cm for 24 h; 2023 MHz at 190 mV/cm for 24 h; 2083 MHz at 190 mV/cm for 56 h; 2103 MHz at 190 mV/cm for 56 h.

The activity of the activated yeast cells towards dichlorvos was determined by measuring the amounts of dichlorvos the activated yeast cells can degrade. Two 100 liter

samples each containing dichlorvos concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of dichlorvos remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dichlorvos in the samples with the activated yeast cells was reduced by more than 67.5%.

Yeast cell component that decomposes Momocrotophos

In a specific embodiment, a method for producing yeast cells that decompose momocrotophos and related insecticides is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.93 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 2998 MHz at 165 mV/cm for 24 h; 2033 MHz at 165 mV/cm for 24 h; 2058 MHz at 165 mV/cm for 24 h; 2113 MHz at 165 mV/cm for 24 h; 2998 MHz at 202 mV/cm for 56 h; 2033 MHz at 202 mV/cm for 56 h; 2058 MHz at 202 mV/cm for 24 h; 2113 MHz at 202 mV/cm for 24 h.

The activity of the activated yeast cells towards momocrotophos was determined by measuring the amount of momocrotophos the activated yeast cells can degrade. Two 100 liter samples each containing momocrotophos concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of monocrotophos remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of momocrotophos in the samples with the activated yeast cells was reduced by more than 73.4%.

Yeast cell component that decomposes Dimethoate

In a specific embodiment, a method for producing yeast cells that decompose dimethoate and related insecticidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1983.000 to 2118.000 including but not limited to 1983, 1988, 1993, 1998, 2003, 2008, 2013, 2018, 2023, 2028, 2033, 2038, 2043, 2048, 2053, 2058, 2063, 2068, 2073, 2078, 2083, 2088, 2093, 2098, 2103, 2108, 2113, and 2118 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.379 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1988 MHz at 195 mV/cm for 24 h; 2023 MHz at 195 mV/cm for 24 h; 2088 MHz at 195 mV/cm for 24 h; 2108 MHz at 195 mV/cm for 24 h; 1988 MHz at 277 mV/cm for 56 h; 2023 MHz at 277 mV/cm for 56 h; 2088 MHz at 277 mV/cm for 24 h; 2108 MHz at 277 mV/cm for 24 h.

The activity of the activated yeast cells towards dimethoate was determined by measuring the amount of dimethoate the activated yeast cells can degrade. Two 100 liter samples each containing dimethoate concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of dimethoate remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of dimethoate in the samples with the activated yeast cells was reduced by more than 69.6%.

Yeast cell component that decomposes DDT

In a specific embodiment, a method for producing yeast cells that decompose DDT and related dilorinated organic insecticidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1420.000 to 1435.000 including but not limited to 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.415 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 8 EM fields in the order stated: 1423 MHz at 75 mV/cm for 24 h; 1426 MHz at 75 mV/cm for 24 h; 1433 MHz at 75 mV/cm for 24 h; 1435 MHz at 75 mV/cm for 24 h; 1423 MHz at 146 mV/cm for 56 h; 1426 MHz at 146 mV/cm for 56 h; 1433 MHz at 146 mV/cm for 24 h; 1435 MHz at 146 mV/cm for 24 h.

The activity of the activated yeast cells towards DDT was determined by measuring the amount of DDT the activated yeast cells can degrade. Two 100 liter samples each containing DDT concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of DDT remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of DDT in the samples with the activated yeast cells was reduced by more than 78.5%.

Yeast cell component that decomposes Toxaphene

In a specific embodiment, a method for producing yeast cells that decompose toxaphene and related chlorinated organic insecicidal compounds is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1420.000 to 1435.000 including but not limited to 1420, 1421, 1422, 1423, 1424, 1425, 1426, 1427, 1428, 1429, 1430, 1431, 1432, 1433, 1434, 1435 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.504 are cultured at about 25-30°C in a culture medium as described in Table 2 in the presence of a series of 4 EM fields in the order stated: 1420 MHz at 120 mV/cm for 24 h; 1426 MHz at 120 mV/cm for 24 h; 1431 MHz at 120 mV/cm for 24 h; 1434 MHz at 120 mV/cm for 24 h.

The activity of the activated yeast cells towards toxaphene was determined by measuring the amount of toxaphene the activated yeast cells can degrade. Two 100 liter samples each containing toxaphene concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of toxaphene remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of toxaphene in the samples with the activated yeast cells was reduced by more than 70.8%.

5.3. ODOR-REDUCING YEAST CELL COMPONENTS

The present invention also provides yeast cells that are capable of reducing the odor of solid waste, e.g., manures, sludge, and/or garbage. Without being bound by any theory, the inventor believes that the yeast cells of the invention are capable of reducing the

odor of solid waste by modifying, assimilating, or decomposing known and unknown compounds in the solid waste that are malodorous. However, it is not necessary to demonstrate that such compounds have been decomposed. It is sufficient so long as the odor is reduced as determined subjectively by a panel of subjects, after the yeast cells of the invention have been used.

According to the present invention, yeast cells that are capable of reducing the odor of solid waste are prepared by culturing the cells in the presence of an electromagnetic field in an appropriate culture medium. The frequency of the electromagnetic field for activating or enhancing this ability in yeasts can generally be found in the range of 2160 to 2380 MHz. After sufficient time is given for the yeast cells to grow, the yeast cells can be tested for their ability to reduce the odor of solid waste by methods well known in the art.

The method of the invention for making the odor-reducing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(NH_4)_2HPO_4$, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

It should be noted that the composition of the media provided in Table 38 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2-10^5 cell/ml, preferably $3x10^2$-10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the

same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 2160 to 2380 MHz, and preferably in the ranges of 2160.000 to 2250.000 MHz or 2280.000 to 2380.000 MHz. The field strength of the EM field(s) is in the range of 25 to 300 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 80-320 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of 25-200 mV/cm. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 250-300mV/cm. The process of the invention is carried out at temperatures ranging from about 25° to 30°C; however, it is preferable to conduct the process at 28°C. The culturing process may preferably be conducted under conditions in which the concentration of dissolved oxygen is between 0.025 to 0.8 mol/m^3, preferably 0.4 mol/m^3. The oxygen level can be controlled by any conventional means known to one skilled in the art, including but not limited to stirring and/or bubbling.

At the end of the culturing process, the yeast cells may be recovered from the culture by various methods known in the art, and stored at a temperature below about 0-4°C. The recovered yeast cells may also be dried and stored in powder form.

Any methods known in the art can be used to test the cultured yeast cells for their ability to reduce the odor of organic materials. The amount of malodorous chemicals such as hydrogen sulfide, ammonia, indole, p-cresol, skatol, and organic acids present in a test sample of organic material can be determined by any methods known in the art,

including but not limited to gas phase chromatography, olfactometry, mass spectrometry, or the use of an odor panel.

For example, to determine the activity of the activated yeast cells towards an malodorous compound, mass spectrometry (e.g., VG micromass) can be used to measure the amounts of the malodorous compound in a test sample at various time point and under different incubation conditions. For example, a known amount of a malodorus compound (up to 100 mg per liter) is added to 10 liter of an aqueous extract of manure. Then, 0.1 ml of activated and unactivated yeasts (at least 10^7 cells/ml) are added to the 10 liter samples containing the compound, and incubated for 24 hours at 28°C. A control is included which does not contain any yeast cells. After 24 hours, the amounts of the malodorous compounds remaining in the extracts are determined and compared.

Yeast cell component that reduce odor caused by sulfur containing compounds

In one embodiment of the invention, a method for producing yeast cells that remove hydrogen sulfide and other related sulfur-containing or sulfhydryl (SH-) containing molecules is provided. Yeast cells that remove hydrogen sulfide and other related sulfur-containing or sulfhydryl (SH-) containing molecules can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.559 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2165 MHz at 240 mV/cm for 20 hr; 2175 MHz at 240 mV/cm for 20-60 hr; 2200 MHz at 240 mV/cm for 20 hr; 2235 MHz at 240 mV/cm for 20 hr.

The activity of the activated yeast cells towards sulfur-containing or sulfhydryl (SH-) compounds was determined by measuring the change in amount of hydrogen sulfides in the presence of the activated yeast cells. Two 100 liter samples each containing hydrogen sulfide concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of hydrogen sulfide remaining in the samples were determined and compared. Comparing to

samples with unactivated yeast cells, the amount of hydrogen sulfide in the samples with the activated yeast cells was reduced by more than 59.8%.

Yeast cell component that reduce odor caused by NH-containing containing compounds

In another embodiment of the invention, a method for producing yeast cells that remove ammonia and related NH-containing compounds is provided. Yeast cells that remove ammonia and related NH-containing compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.423 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2160 MHz at 250 mV/cm for 20 hr; 2175 MHz at 250 mV/cm for 20 hr; 2210 MHz at 250 mV/cm for 20 hr; 2245 MHz at 250 mV/cm for 10 hr.

The activity of the activated yeast cells towards ammonia acid NH-containing compounds was determined by measuring the change in the amount of ammonia in the presence of the activated yeast cells. Two 100 liter samples each containing NH-containing compounds at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of ammonia remaining in the samples were determined and compared. Comparing to samples with unactivated yeast cells, the amount of ammonia in the samples with the activated yeast cells was reduced by more than 69.6%.

Yeast cell component that reduce odor caused by indole and other related compounds

In the invention, a method for producing yeast cells that decompose indole and other related compounds, such as skatol is provided. Yeast cells that decompose indole and other related compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.612 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2165 MHz at 240 mV/cm for 40 hr; 2180 MHz at 240 mV/cm for 20 hr; 2200 MHz at 240 mV/cm for 40 hr; 2220 MHz at 240 mV/cm for 20 hr.

The activity of the activated yeast cells towards indole and other related compounds was determined by measuring the amount of indole removed by the activated yeast cells. Two 100 liter samples each containing indole related compounds at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of indole remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of indole in the samples with the activated yeast cells was reduced by more than 71.3%.

Yeast cell component that reduce odor caused by organic acids

In yet another embodiment of the invention, a method for producing yeast cells that remove odorous organic acids, e.g., formic acid, acetic acid, propanoic acid, butyric acid, and other volatile fatty acids, is provided. Yeast cells that can reduce the odor of organic acids can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.53 are cultured at about 25-30°C in a culture medium as described in Table 42 in the presence of a series of 4 EM fields in the order stated: 2315 MHz at 290 mV/cm for 30 hr; 2335 MHz at 290 mV/cm for 10 hr; 2355 MHz at 290 mV/cm for 20 hr; 2375 MHz at 290 mV/cm for 10 hr.

The activity of the activated yeast cells towards organic acids was determined by measuring the change in the amounts of acetic acid in the presence of the activated yeast cells. Two 100 liter samples each containing organic acids concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and

incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of acetic acid remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of acetic acid in the samples with the activated yeast cells was reduced by more than 89.4%.

Yeast cell component that reduce odor caused by aliphatic substituted amine

In yet another embodiment of the invention, a method for producing yeast cells that remove or degrade aliphatic substituted amine, such as methylamine, dimethylamine, or trimethylamine thereby reducing the odor caused by such compounds, is provided. Yeast cells that remove or degrade such amines can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.541 are cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2160 MHz at 250 mV/cm for 20 hr; 2190 MHz at 250 mV/cm for 10 hr; 2210 MHz at 250 mV/cm for 40 hr; 2250 MHz at 250 mV/cm for 40 hr.

The activity of the activated yeast cells towards methyl-substituted amine was determined by measuring the amount of such amine in the presence of the activated yeast cells. Two 100 liter samples each containing methyl-substituted amine at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amount of methyl-substituted amines remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of methyl-substituted amines in the samples with the activated yeast cells was reduced by more than 82.2%.

Yeast cell component that reduce odor caused by p-cresol and related compounds

In yet another embodiment of the invention, a method for producing yeast cells that remove or degrade p-cresol and related compounds is provided. Yeast cells that

remove or degrade p-cresol and other related compounds can be prepared by culturing the cells in the presence of an EM field that is in the range of 2160.000 to 2250.000 MHz, including but not limited to 2160, 2165, 2170, 2175, 2180, 2185, 2190, 2195, 2200, 2205, 2210, 2215, 2220, 2225, 2230, 2235, 2240, 2245, and 2250 MHz.

For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.163 were cultured at about 25-30°C in a culture medium as described in Table 3 in the presence of a series of 4 EM fields in the order stated: 2300 MHz at 98 mV/cm for 20 hr; 2370 MHz at 98 mV/cm for 15 hr; 2300 MHz at 250 mV/cm for 20 hr; 2370 MHz at 250 mV/cm for 30 hr.

The activity of the activated yeast cells towards p-cresol and related compounds was determined by measuring the change in the amounts of p-cresol and related compounds in the presence of the activated yeast cells. Two 100 liter samples each containing p-cresol at a concentration of 100mg/L were prepared. Then, 0.1 ml of activated yeast cells and 0.1 ml of unactivated yeasts cells (both containing 10^7 cells/ml) were added separately into the two samples, and incubated for 24 hours at 28°C. A control was included which did not contain any yeast cells. After 24 hours, the amounts of p-cresol remaining in the samples were determined and compared by performing HPLC on the samples. Comparing to samples with unactivated yeast cells, the amount of p-cresol in the samples with the activated yeast cells was reduced by more than 92.5%.

5.4. PATHOGEN-SUPPRESSING YEAST CELL COMPONENTS

The present invention also provides yeast cells that are capable of suppressing the proliferation of pathogenic microorganisms that are present in solid waste. Typically, due to an abundance of nutrients present in solid waste for such pathogenic microorganisms, the numbers of pathogens increase rapidly over a period of time. However, in the presence of the pathogen-suppressing yeasts of the invention, the numbers of pathogens in the treated solid waste remains unchanged, or decreases over time. Without being bound by any theory or mechanism, the inventor believes that the presence of the pathogen-suppressing yeasts in the solid waste creates an environment that is unfavorable for the growth of pathogenic microorganisms.

According to the invention, the ability of yeasts to affect/control the numbers of pathogens is activated or enhanced by culturing the yeasts in the presence of an electromagnetic field. The resulting pathogen-suppressing yeast cells are used as a component in the solid waste treatment compositions of the invention.

The frequency of the electromagnetic field for activating or enhancing the ability of yeasts to control the numbers of pathogenic microorganisms can generally be

found in the range of 30 MHz to 50 MHz. After sufficient time is given for the yeast cells to grow, the cells can be tested for their ability to affect/control the number of pathogens by methods well known in the art.

The method of the invention for making pathogen-suppressing yeast cells is carried out in a liquid medium. The medium contains sources of nutrients assimilable by the yeast cells. In general, carbohydrates such as sugars, for example, sucrose, glucose, fructose, dextrose, maltose, xylose, and the like and starches, can be used either alone or in combination as sources of assimilable carbon in the culture medium. The exact quantity of the carbohydrate source or sources utilized in the medium depends in part upon the other ingredients of the medium but, in general, the amount of carbohydrate usually varies between about 0.1% and 5% by weight of the medium and preferably between about 0.5% and 2%, and most preferably about 0.8%. These carbon sources can be used individually, or several such carbon sources may be combined in the medium.

Among the inorganic salts which can be incorporated in the culture media are the customary salts capable of yielding sodium, calcium, phosphate, sulfate, carbonate, and like ions. Non-limiting examples of nutrient inorganic salts are $(NH_4)_2HPO_4$, $CaCO_3$, $MgSO_4$, NaCl, and $CaSO_4$.

Table 4: Composition for a culture medium for Pathogen-Suppressing yeasts

Medium Composition	Quantity
Soluble Starch	8.0g
Sucrose	5g
NaCl	0.2g
$MgSO_4 \cdot 7H_2O$	0.2g
$CaCO_3 \cdot 5H_2O$	0.5g
$CaSO_4 \cdot 2H_2O$	0.2g
Peptone	1.5g
K_2HPO_4	0.5g
Autoclaved water	400ml
Pathogen extract	600ml

The pathogen extract for the culture medium is prepared by incubating 500g of pathogen-containing waste in about 600ml of warm water (at 35°C to 40°C) for 24 hours

at 30-37°C, and filtering the fluid to remove particulate matters. It should be noted that the composition of the media provided in Table 4 is not intended to be limiting. Various modifications of the culture medium may be made by those skilled in the art, in view of practical and economic considerations, such as the scale of culture and local supply of media components.

The process can be initiated by inoculating 100ml of medium with 1ml of an inoculum of the selected yeast strain(s) at a cell density of 10^2-10^5 cell/ml, preferably $3x10^2$-10^4 cell/ml. The process can be scaled up or down according to needs. The yeast culture is grown in the presence of an electromagnetic (EM) field, or a series of EM fields. If a series of EM fields are applied, the yeast culture can remain in the same container and use the same set of electromagnetic wave generator and emitters when switching from one EM field to another EM field.

The EM field(s), which can be applied by any means known in the art, can each have a frequency in the range of 30.000 to 50.000, 500.000 to 650.000, and 1000.000 to 1150.000 MHz. The field strength of the EM field(s) is in the range of 20 to 200 mV/cm. If a series of EM fields are applied, the EM fields can each have a different frequency within the stated range, or a different field strength within the stated range, or different frequency and field strength within the stated ranges. In a preferred embodiment, the EM field(s) at the beginning of a series have a lower EM field strength than later EM field(s), such that the yeast cell culture are exposed to EM fields of progressively increasing field strength. Although any practical number of EM fields can be used within a series, it is preferred that the yeast culture be exposed to a total of 2, 3, 4, 5, 6, 7, 8, 9 or 10 different EM fields in a series.

Although the yeast cells will become activated even after a few hours of culturing in the presence of the EM field(s), and the yeast cells can be cultured in the presence of the EM field(s) for an extended period of time (e.g., two or more weeks), it is generally preferred that the activated yeast cells be allowed to multiply and grow in the presence of the EM field or EM fields for a total of about 144 - 272 hours.

For example, using an exemplary apparatus as depicted in Figure 1, an output amplitude of the EM wave in the range of 20-180 mV/cm. After this first period of culture, the yeast cells are further incubated under substantially the same conditions for another period, except that the amplitude is increased to a higher level in the range of 200-350 mV/cm.

At the end of the culturing process, the pathogen-suppressing yeast cells may be recovered from the culture by various methods known in the art, and stored at about 0°C to 4°C. The pathogen-suppressing yeast cells may also be dried and stored in powder form.

The ability of the pathogen-suppressing yeasts to control the numbers of pathogens can be determined by any methods known in the art for enumerating microorganisms, such as optical density, plating out dilutions on solid media for counting, or counting individual cells under a microscope. Stains may be applied to distinguish or identify different strains or species of microorganisms present in a sample, or to determine their viability. When a range of pathogenic microorganisms are expected to be affected by the pathogen-suppressing yeasts, the numbers of more than one representative species of pathogenic microorganisms can be monitored to assess the performance of the pathogen-suppressing yeasts.

For example, samples of solid waste containing a known concentration of pathogenic microorganisms are cultured under the same conditions for a same period of time in the presence of different concentrations of pathogen-suppressing yeasts, and as negative control, the same strain of yeasts that have not been treated according to the culturing methods of the invention. A sample without any added yeast may also be included to determine the growth of pathogens under normal circumstances. The numbers of pathogens before and after the culture period are determined and compared.

A one liter culture containing at least 10^{10} cells of a pathogenic microorganism per ml is prepared. One ml of activated yeast cells (containing 2 to 5 x 10^7 yeasts per ml) is added to the one liter culture of pathogenic microorganism and incubated at 30°C for 24 hours. A control is included which contained unactivated yeast cells. The numbers of microorganisms in the respective culture is then determined and compared. The following are several examples of which a particular species of pathogenic bacteria was studied.

Yeast cell component that suppresses *Staphylococcus aureus*

In a specific embodiment of the invention embodiment, a method for producing yeast cells that suppress the growth of *Staphylococcus aureus* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.595 were cultured at about 25-30°C in a culture medium as described

in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12h; 43 MHz at 26 mV/cm for 12 h; 47 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 43 MHz at 150 mV/cm for 24 h; 47 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *Staphylococcus aureus* was determined by measuring the growth of *Staphylococcus aureus* in the presence of the activated yeast cells. *Staphylococcus aureus* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Staphylococcus aureus* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.6%.

Yeast cell component that suppresses *Diplococcus pneumoniae*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *Diplococcus pneumoniae* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain IFFI1021 were cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12h; 42 MHz at 26 mV/cm for 12 h; 49 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 42 MHz at 150 mV/cm for 24 h; 49 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *Diplococcus pneumoniae* was determined by measuring the growth of *Diplococcus pneumoniae* in the presence of the activated yeast cells. *Diplococcus pneumoniae* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Diplococcus pneumoniae* in the samples were determined by conventional

bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 3 %.

Yeast cell component that suppresses *Bacillus anthracis*

In a specific embodiment, a method for producing yeast cells that suppress *the growth of Bacillus anthracis* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 20.000 to 45.000 MHz, including but not limited to 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, and 45 MHz. For example, yeast cells of *Bacillus anthracis* strain AS2.390 are cultured at about 25-30 °C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 24 MHz at 100 mv/cm for 24 h, 37 MHz at 100 mv/cm for 24 h, 40 MHz at 100 mv/cm for 24 h, 45 MHz at 100 mv/cm for 24 h, 24 MHz at 190 mv/cm for 24 h, 37 MHz at 190 mv/cm for 24 h, 40 MHz at 190 mv/cm for 24 h, 45 MHz at 190 mv/cm for 24 h.

The activity of the activated yeast cells towards *Bacillus anthracis* was determined by measuring the growth of *Bacillus anthracis* in the presence of the activated yeast cells. *Bacillus anthracis* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Bacillus anthracis* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.6 %.

Yeast cell component that suppresses *Mycobacterium tuberculosis*

In a specific embodiment, a method for producing yeast cells that suppress *Mycobacterium tuberculosis* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.431 are cultured at about 25-30 ° in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 33 MHz at 26 mv/cm for 12 h; 36 MHz at 26 mv/cm for 12 h; 45 MHz

at 26 mv/cm for 12 h; 47 MHz at 26 mv/cm for 12 h; 33 MHz at 150 mv/cm for 24 h; 36 MHz at 150 mv/cm for 24 h; 45 MHz at 150 mv/cm for 24 h; 47 MHz at 150 mv/cm for 24 h.

The activity of the activated yeast cells towards *Mycobacterium tuberculosis* was determined by measuring the growth of *Mycobacterium tuberculosis* in the presence of the activated yeast cells. *Mycobacterium tuberculosis* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Mycobacterium tuberculosis* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 2.9 %.

Yeast cell component that suppresses *E. coli*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *E. coli* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.561 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 34 MHz at 26 mV/cm for 12 h; 38 MHz at 26 mV/cm for 12 h; 49 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 34 43 MHz at 150 mV/cm for 24 h; 38 MHz at 150 mV/cm for 24 h; 49 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *E. coli* was determined by measuring the growth of *E. coli* in the presence of the activated yeast cells. *E. coli* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *E. coli* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with

unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 48%.

Yeast cell component that suppresses *Salmonella*

In a specific embodiment, a method for producing yeast cells that suppress the growth of *Salmonella* species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 30.000 to 50.000 MHz, including but not limited to 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.178 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 30 MHz at 26 mV/cm for 12 h; 33 MHz at 26 mV/cm for 12 h; 36 MHz at 26 mV/cm for 12 h; 38 MHz at 26 mV/cm for 12 h; 30 MHz at 150 mV/cm for 24 h; 33 MHz at 150 mV/cm for 24 h; 36 MHz at 150 mV/cm for 24 h; 38 MHz at 150 mV/cm for 24 h.

The activity of the activated yeast cells towards *Salmonella* species was determined by measuring the growth of *Salmonella* in the presence of the activated yeast cells. *Salmonella* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Salmonella* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 62 %.

Yeast cell component that suppresses Vibrio species

In a specific embodiment, a method for producing yeast cells that suppress Vibrio species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of to 500.000 to 550.000 MHz, including but not limited to 520, 521, 522, 523, 524, 525, 526, 527, 528, 529, 530, 531, 532, 533, 534, 535, 536, 537, 538, 539, and 540 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.377 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 521 MHz at 150 mv/cm for 24 h, 527 MHz at 150

mv/cm for 24 h, 531 MHz at 150 mv/cm for 24 h, 538 MHz at 150 mv/cm for 24 h, 521 MHz at 276 mv/cm for 24 h, 527 MHz at 276 mv/cm for 24 h, 531 MHz at 276 mv/cm for 24 h, 538 MHz at 276 mv/cm for 24 h.

The activity of the activated yeast cells towards *Vibrio* species was determined by measuring the growth of *Vibrio* species in the presence of the activated yeast cells. *Vibrio* species contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Vibrio* species in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 5.6 %.

Yeast cell component that suppresses Shigella species

In a specific embodiment, a method for producing yeast cells that suppress Shigella species is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 600.000 to 650.000 MHz, including but not limited to 630, 631, 632, 633, 634, 635, 636, 637, 638, 639, 640, 641, 642, 643, 644, 645, 646, 647, 648, 649, and 650 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.395 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 630 MHz at 180 mv/cm for 24 h, 636 MHz at 180 mv/cm for 24 h, 641 MHz at 180 mv/cm for 24 h, 649 MHz at 180 mv/cm for 24 h, 630 MHz at 314 mv/cm for 24 h, 636 MHz at 314 mv/cm for 24 h, 641 MHz at 314 mv/cm for 24 h, 649 MHz at 314 mv/cm for 24 h.

The activity of the activated yeast cells towards *Shigella* species was determined by measuring the growth of *Shigella* species in the presence of the activated yeast cells. *Shigella* species contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Shigella* species in the samples were determined by conventional bacteria cell counting

method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 4.6 %.

Yeast cell component that suppresses *Clostridium botulinum*

In a specific embodiment, a method for producing yeast cells that suppress *Clostridium botulinum* is provided. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1000.000 to 1050.000 MHz, including but not limited to 1010, 1011, 1012, 1013, 1014, 1015, 1016, 1017, 1018, 1019, 1020, 1021, 1022, 1023, 1024, 1025, 1026, 1027, 1028, 1029, 1030, 1031, 1032, 1033, 1034, and 1035 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.432 are cultured at about 25-30°C in a culture medium as described in Table 4 in the presence of a series of 8 EM fields in the order stated: 1012 MHz at 180 mv/cm for 24 h, 1018 MHz at 180 mv/cm for 24 h, 1024 MHz at 180 mv/cm for 24 h, 1033 MHz at 180 mv/cm for 24 h, 1012 MHz at 323 mv/cm for 24 h, 1018 MHz at 323 mv/cm for 24 h, 1024 MHz at 323 mv/cm for 24 h, 1033 MHz at 323 mv/cm for 24 h.

The activity of the activated yeast cells towards *Clostridium botulinum* was determined by measuring the growth of *Clostridium botulinum* in the presence of the activated yeast cells. *Clostridium botulinum* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Clostridium botulinum* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 5.1 %.

Yeast cell component that suppresses *Bacillus aerogenes capsulatus*

In a specific embodiment, a method for producing yeast cells that suppress *Bacillus aerogenes capsulatus*. The frequencies of the EM field(s) used to activate the yeast cells are in the range of 1100.000 to1150.000 MHz, including but not limited to 1100, 1101, 1102, 1103, 1104, 1105, 1106, 1107, 1108, 1109, 1110, 1111, 1112, 1113, 1114, 1115, 1116, 1117, 1118, 1119, and 1120 MHz. For example, yeast cells of *Saccharomyces cerevisiae* strain AS2.432 are cultured at about 25-30°C in a culture medium as described in

Table 4 in the presence of a series of 8 EM fields in the order stated: 1102 MHz at 180 mv/cm for 24 h, 1106 MHz at 180 mv/cm for 24 h, 1113 MHz at 180 mv/cm for 24 h, 1117 MHz at 180 mv/cm for 24 h, 1102 MHz at 301 mv/cm for 24 h, 1106 MHz at 301 mv/cm for 24 h, 1113 MHz at 301 mv/cm for 24 h, 1117 MHz at 301 mv/cm for 24 h.

The activity of the activated yeast cells towards *Bacillus aerogenes capsulatus* was determined by measuring the growth of *Bacillus aerogenes capsulatus* in the presence of the activated yeast cells. *Bacillus aerogenes capsulatus* contained in an extract from solid waste was grown in a culture until the cell count reached 10^{10} cells/ml. 1 ml of activated yeast cells and 1 ml of unactivated yeasts cells (both containing about 10^7 cells/ml) were added separately into two 1-liter samples of the bacterial culture, and incubated for 24 hours at 20-37°C. A control was included which did not contain any yeast cells. After 24 hours, the cell count of *Bacillus aerogenes capsulatus* in the samples were determined by conventional bacteria cell counting method. Comparing to samples with unactivated yeast cells, the cell count in the samples with the activated yeast cells was reduced by 6.2 %.

5.5 ADAPTATION

In another embodiment of the invention, activated yeast cells prepared according to any one of Sections 5.1-5.10 can be further cultured as a mixture in the presence of a sample of the solid waste which is to be treated. This optional process which improves the performance of the solid waste treatment compositions is described by way of an example as follows.

An extract of the solid waste to be treated, such as manure or sludge, is prepared by mixing and soaking about 1000 g of poultry manure in 1000 to 3000 ml of water for about 48 hours. The extract is then mixed with about 1000 g of dried manure (dry weight, i.e., less than 10% moisture) to form a suspension to which the yeast cells are added. At least 1 ml of yeasts which contains more than $5x10^7$ cell/ml is added to the suspension. Depending on the number of strains of activated yeast cells used, up to about 50 ml of yeast cells can be added. If only a few strains are used, 5 to 10ml of yeast cells per strain can be added. The process can be scaled up or down according to needs. The mixture of yeast and solid waste is cultured for about 120-280 hours in the presence of a series of electromagnetic fields. Each electromagnetic field has a frequency that, depending on the strains of yeast included, corresponds to one of the frequencies described in Sections 5.1-5.4. If many different strains of yeasts are used, a combination of the following five frequency bands can be used : 20-50 MHz, 60-150 MHz, 400-700 MHz, 1400-1600 MHz,

2000-2500 MHz; each for about 24 to 56 hours. Generally, the yeast cells are subjected to an EM field strength in the range from 20mV/cm to 350mV/cm in this process.

The culture is incubated at temperatures that cycle between about 5°C to about 37°C. For example, in a typical cycle, the temperature of the culture may start at about 37°C and be kept at this temperature for about 1-2 hours, then adjusted to 26-30°C and kept at this temperature for about 2-4 hours, and then brought down to 5 -10°C and kept at this temperature for about 1-2 hours, and then the temperature may be raised again to about 37°C for another cycle. The cycles are repeated until the process is completed. After the last temperature cycle is completed, the temperature of the culture is lowered to 3-4°C and kept at this temperature for about 5-6 hours. After the process, the yeast cells may be isolated and recovered from the medium by conventional methods, such as filtration. The adapted yeast cells can be stored under 4°C. An exemplary set-up of the culture process is depicted in Figure 2.

5.6 MANUFACTURE OF THE BIOLOGICAL COMPOSITIONS

The biological composition of the present invention can be produced by culturing yeast cells under appropriate conditions according to Section 5.1 to 5.4, and mixing the desired amounts of cultures of yeast cells. Since the biological composition is not immediately used to treat solid waste, the yeasts of the biological composition can be dried in a two-stage drying process. During the first drying stage, the yeast cells are dried in a first dryer at a temperature not exceeding 65°C for a period of time not exceeding 10 minutes so that yeast cells quickly become dormant. The yeast cells are then sent to a second dryer and dried at a temperature not exceeding 70°C for a period of time not exceeding 30 minutes to further remove water. After the two stages, the water content should be lower than 5%. It is preferred that the temperatures and drying times be adhered to in both drying stages so that yeast cells do not lose their vitality and functions. The dried yeast cells are then cooled to room temperature. The dried yeast cells may also be screened in a separator so that particles of a preferred size are selected. The dried cells can then be sent to a bulk bag filler for packing.

The present invention is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the invention, and functionally equivalent methods and components are within the scope of

the invention. Indeed various modifications of the invention, in addition to those shown and described herein will become apparent to those skilled in the art from the foregoing description and accompanying drawings. Such modifications are intended to fall within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A method for treatment of solid waste comprising antibiotics, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the antibiotics, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 100 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of penicillin, chlortetracycline, oxytetracycline, doxycycline, tetracycline, streptomycin, kanamycin, erythromycin, spiramycin and bacitracin;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 410 to 470 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of colistin, chloramphenicol, and cephalothin; or

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 550 to 620 MHz and a field strength of 8.5 to 250 mV/cm, and which can degrade antibiotics selected from the group consisting of neomycin and novobiocin.

2. A method for treatment of solid waste comprising undesirable chemicals , said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following::

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 52 to 98 MHz and a field strength of 8 to 300 mV/cm, which can degrade toluene, ethylbenzene, or trichlorophenol;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 30 to 50 MHz or 70 to 98 MHz and a field strength of 8 to 250 mV/cm; and which can degrade dimethylbenezene compounds;

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 133 to 151 MHz and a field strength of 8 to 250 mV/cm, and which can degrade benzaldehyde;

(d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 145 to 162 MHz and a field strength of 8 to 250 mV/cm, and which can degrade propylaldehyde;

(e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 100 MHz and a field strength of 8 to 250 mV/cm, and which can degrade nenanthaldehyde; and

(f) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 163 to 183 MHz and a field strength of 8 to 250 mV/cm, and which can degrade dichlorobenzene.

3. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 175 to 191 MHz and a field strength of 8 to 250 mV/cm, and which can degrade acetophenone;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 183 to 205 MHz and a field strength of 8 to 250 mV/cm, and which can degrade arsanilic acid;

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 114 to 128 MHz and a field strength of 8 to 250 mV/cm, and which can degrade is roxarsone;

(d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of

70 to 98 MHz or 200 to 220 MHz and a field strength of 8 to 250 mV/cm and which can degrade furazolidonum;

(e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 213 to 229 MHz and a field strength of 8 to 250 mV/cm and which can degrade decoquinate; and

(f) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 70 to 98 MHz or 220 to 250 MHz and a field strength of 8 to 250 mV/cm, and which can degrade trichlorophonum or dinitomide.

4. A method for treatment of solid waste comprising undesirable chemicals, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 660 to 680 MHz or 2160 to 2190 MHz and a field strength of 25 to 300 mV/cm, and which can reduce the amount of ammonium compounds;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 661 to 680 MHz and a field strength of 8 to 250 mV/cm, and which can reduce the amount of nitrites or nitrates; or

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 86 to 120 MHz or 410 to 430 MHz and a field strength of 8 to 250 mV/cm, and which can reduce the amount of phosphates.

5. A method for treatment of solid waste comprising undesirable chemicals , said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to degrade the undesirable chemicals, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1980 to 2118 MHz and a field strength of 25 to 300mV/cm, and which can

degrade undesirable chemical selected from the group consisting of trichlorphon, dichlorvos, momocrotophos and dimethoate;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1420 to 1435 MHz and a field strength of 25 to 300mV/cm, and which can degrade DDT or toxaphene.

6. A method for reducing the odor of solid waste comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to reduce the amount of odorous molecules in the solid waste, wherein said yeast are prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 2160 to 2380 MHz and a field strength of 25 to 300 mV/cm, and said odorous molecules are selected from the group consisting of hydrogen sulfide, ammonia, indole, skatol, acetic acid, methylamine, and p-cresol.

7. A method for treatment of solid waste comprising pathogenic bacteria, said method comprising adding a plurality of yeast cells to the solid waste and, allowing the yeast cells to suppress the growth of pathogenic bacteria in the solid waste, wherein said plurality of yeast cells comprises at least one of the following:

(a) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 20 to 50 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of Staphylococcus aureus, Diplococcus pneumoniae, Bacillus anthracis, Mycobacterium tuberculosis, Salmonella species, or E. coli;

(b) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 500 to 550 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of Vibrio species;

(c) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 600 to 650 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of Shigella species;

(d) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of

1000 to 1050 MHz and a field strength of 20 to 350 mV/cm, and which can suppress the growth of Clostridium botulinum; and

(e) yeast cells prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 1100 to 1150 MHz and a field strength of 20 to 50 mV/cm, and which can suppress the growth of Bacillus aerogenes capsulatus.

8. A method for treatment of solid waste comprising adding a biological composition to the solid waste, said biological composition comprising at least one of the following yeast cell components:

(a) a first yeast cell component comprising a plurality of yeast cells that degrade antibiotics in solid waste, said first yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 70 to 100 MHz, 410 to 470 MHz, and 550 to 620 MHz and a field strength of 8 to 250 mV/cm;

(b) a second yeast cell component comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste, said second yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 30 to 100 MHz, 70 to 280 MHz, 410 to 430 MHz, 660 to 680 MHz and 1980 to 2210 MHz and a field strength of 8 to 250 mV/cm;

(c) a third yeast cell component comprising a plurality of yeast cells that reduce the amount of odorous molecules in solid waste, said third yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range of 2160 to 2380 MHz and a field strength of 25 to 300 mV/cm;

(d) a fourth yeast cell component comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in the solid waste, said fourth yeast cell component being prepared by culturing the yeast cells in an electromagnetic field or a series of electromagnetic fields having a frequency in the range selected from the group consisting of 30 to 50 MHz, 500 to 550 MHz, 600 to 650 MHz, 1000 to 1050 MHz, and 1100 to 1150 MHz and a field strength of 20 to 350 mV/cm; and

allowing the yeast cells in the yeast cell component(s) to reduce the amount of antibiotics, undesirable chemicals, odorous compounds and pathogenic bacteria in the solid waste.

9. The method of claim 8, wherein the biological composition comprises the yeast cell components of (a), (b), (c) and (d).

10. The method of claim 8 wherein said yeast cells are cells of a species of yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces delbrueckii*, *Saccharomyces exiguus*, *Saccharomyces fermentati*, *Saccharomyces logos*, *Saccharomyces mellis*, *Saccharomyces microellipsoides*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum Beijer*, *Saccharomyces willianus*, *Saccharomyces ludwigii*, *Saccharomyces sinenses*, and *Saccharomyces carlsbergensis*.

11. The method of claim 8 wherein said yeast cells are *Saccharomyces cerevisiae* cells.

12. The method of claim 11 wherein said biological composition comprises dried yeast cells, and about 300 to 600g of the biological composition is added per cubic meter of solid waste.

13. The method of claim 11 wherein prior to adding said dried yeast cells to said solid waste, said dried yeast cells are mixed with water in the ratio of about 1000 g yeast cells to about 30 liters, and incubated for about 12 to 24 hours.

14. A composition comprising a plurality of yeast cells that degrade antibiotics in solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 70 to 100 MHz, 410 to 470 MHz, and 550 to 620 MHz and (ii) a field strength of 8 to 250 mV/cm.

15. A composition comprising a plurality of yeast cells that degrade undesirable chemicals in solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 30 to 100 MHz, 70 to 280 MHz, 410 to 430 MHz, 660 to 680 MHz and 1980 to 2210 MHz and (ii) a field strength of 8 to 250 mV/cm.

16. A composition comprising a plurality of yeast cells that reduce the odor of solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range of 2160 to 2380 MHz and (ii) a field strength of 25 to 300 mV/cm;

17. A composition comprising a plurality of yeast cells that suppress the growth of pathogenic bacteria in solid waste, wherein said plurality of yeast cells is prepared by a method comprising culturing yeast cells in an electromagnetic field or a series of electromagnetic fields having (i) one or more frequencies in the range selected from the group consisting of 30 to 50 MHz, 500 to 550 MHz, 600 to 650 MHz, 1000 to 1050 MHz, and 1100 to 1150 MHz and (ii) a field strength of 20 to 350 mV/cm.

18. The composition of claim 14, 15, 16, or 17, wherein said yeast cells are cells of a species of yeast selected from the group consisting of *Saccharomyces cerevisiae*, *Saccharomyces chevalieri*, *Saccharomyces delbrueckii*, *Saccharomyces exiguus*, *Saccharomyces fermentati*, *Saccharomyces logos*, *Saccharomyces mellis*, *Saccharomyces microellipsoides*, *Saccharomyces oviformis*, *Saccharomyces rosei*, *Saccharomyces rouxii*, *Saccharomyces sake*, *Saccharomyces uvarum Beijer*, *Saccharomyces willianus*, *Saccharomyces ludwigii*, *Saccharomyces sinenses*, and *Saccharomyces carlsbergensis*.

19. The composition of claim 14, 15, 16, or 17, wherein said yeast cells are dried yeast cells.

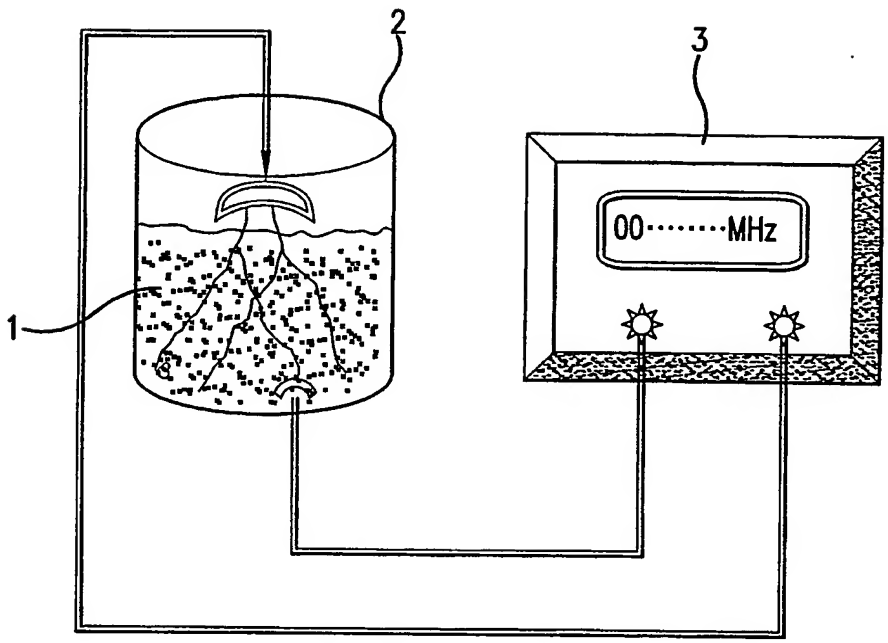


FIG.1

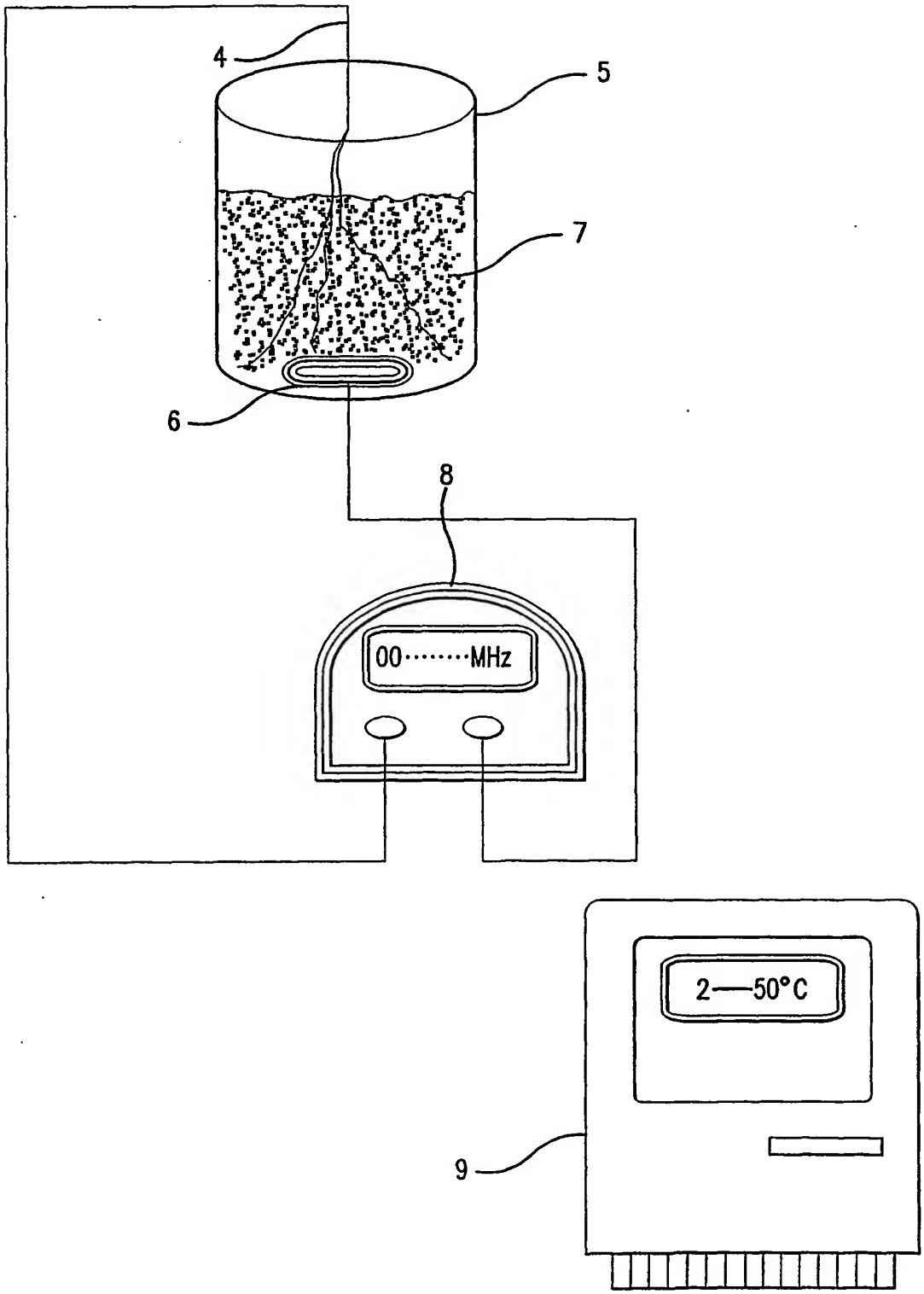


FIG.2

INTERNATIONAL SEARCH REPORT

Interr. , .ial Application No
PCT/GB 02/00915

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N13/00 C12N1/16 C12N1/04 C12P1/02 A62D3/00
B09B3/00 //(C12P1/02,C12R1:645)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C12P C12R

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Saccharomyces cerevisiae Meyen ex Hansen" CHINA CATALOGUE OF CULTURES/CHINA COMMITTEE OF CULTURE COLLECTION FOR MICROORGANISMS (CCCCM), 'Online! 24 April 1996 (1996-04-24), XP002223046 Retrieved from the Internet: <URL:http://www.im.ac.cn/database/YEAST/y122.htm> 'retrieved on 2002-12-02! the whole document ---	14-19
X	WO 87 02705 A (SWEENEY GEORGE WILLIAM JR) 7 May 1987 (1987-05-07) the whole document ---	1-13
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 December 2002	20/12/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Dumont, E

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/00915

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PICHKO V B ET AL: "Electromagnetic stimulation of productivity of microorganisms and its mechanisms." PRIKLADNAYA BIOKHIMIYA I MIKROBIOLOGIYA, vol. 32, no. 4, 1996, pages 468-472, XP009001867 ISSN: 0555-1099 abstract	1-13
X	BINNINGER DAVID M ET AL: "Effects of 60Hz AC magnetic fields on gene expression following exposure over multiple cell generations using Saccharomyces cerevisiae." BIOELECTROCHEMISTRY AND BIOENERGETICS, vol. 43, no. 1, 1997, pages 83-89, XP002223047 ISSN: 0302-4598 the whole document especially abstract; discussion	1-13
X	FR 2 222 433 A (GIANESSY MATILDE) 18 October 1974 (1974-10-18) the whole document	1-13
X	WO 95 04814 A (INT TLB RES INST INC) 16 February 1995 (1995-02-16) page 11, line 25 - line 38 page 16, line 1 - line 10	1-13
X	US 3 870 599 A (AZAROWICZ EDWARD N) 11 March 1975 (1975-03-11) the whole document	15,19
X	DATABASE WPI Week 198439 Derwent Publications Ltd., London, GB; AN 1984-242742 '39! XP002223049 "Yeast strain Exophiala Nigrum R-11 - removes phenol(s) and lignin from aq. effluent(s) " & SU 1 071 637 A (NII BIOLOG PRI IR G UNIV IM A), 7 February 1984 (1984-02-07) abstract	15
E	WO 02 20431 A (ULTRA BIOTECH LTD) 14 March 2002 (2002-03-14) page 8, line 1 -page 13, line 15; claims 1-51	1-19

-/--

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 02/00915

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	PONNE C T ET AL: "Interaction of electromagnetic energy with biological material--relation to food processing" RADIATION PHYSICS AND CHEMISTRY, ELSEVIER SCIENCE PUBLISHERS BV., AMSTERDAM, NL, vol. 45, no. 4, 1 April 1995 (1995-04-01), pages 591-607, XP004051598 ISSN: 0969-806X page 591, column 2, paragraph 3 -page 601, column 2, paragraph 3	1-19

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1, 14 (complete) and 8-13, 18,19 (partial)

   a method for treatment of solid waste comprising antibiotics, comprising adding to the solid waste yeast cells cultured in an electromagnetic field of specific frequency and field strength; a composition comprising such yeasts.

2. Claims: 2, 3, 4, 5, 15 (complete) and 8-13, 18, 19 (partial)

   a method for treatment of solid waste comprising chemicals including herbicides, pesticides and fertilizer-related pollutants, comprising adding to the solid waste yeast cells cultured in an electromagnetic field of specific frequency and field strength; a composition comprising such yeasts.

3. Claims: 6, 16 (complete) and 8-13, 18, 19 (partial)

   a method for reducing the odor of solid waste, comprising adding to the solid waste yeast cells cultured in an electromagnetic field of specific frequency and field strength; a composition comprising such yeasts.

4. Claims: 7,17 (complete) and 8-13, 18, 19 (partial)

   a method of treatment of solid waste comprising pathogenic bacteria, comprising adding to the solid waste yeast cells cultured in an electromagnetic field of specific frequency and field strength; a composition comprising such yeasts.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 02/00915

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 02/00915

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 8702705	A	07-05-1987	AU	6549486 A	19-05-1987
			EP	0244463 A1	11-11-1987
			WO	8702705 A1	07-05-1987
FR 2222433	A	18-10-1974	FR	2222433 A1	18-10-1974
WO 9504814	A	16-02-1995	CN	1081662 A	09-02-1994
			CN	1082016 A	16-02-1994
			CN	1082017 A	16-02-1994
			CN	1103060 A	31-05-1995
			CN	1109595 A	04-10-1995
			CN	1110317 A	18-10-1995
			AU	7630194 A	28-02-1995
			WO	9504814 A1	16-02-1995
US 3870599	A	11-03-1975	CA	978114 A1	18-11-1975
			DE	2127577 A1	09-12-1971
			FR	2095822 A5	11-02-1972
			GB	1353682 A	22-05-1974
			JP	54002788 B	13-02-1979
			US	3856667 A	24-12-1974
			US	3871956 A	18-03-1975
			US	3769164 A	30-10-1973
SU 1071637	A	07-02-1984	SU	1071637 A1	07-02-1984
WO 0220431	A	14-03-2002	US	6416983 B1	09-07-2002
			WO	0220431 A1	14-03-2002
			AU	7020300 A	22-03-2002
			WO	02070436 A2	12-09-2002